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Population Regulation of Mansonia Mosquitoes on  
Water Lettuce (Pistia stratiotes L.)

Annual and Final Report

L. P. Lounibos

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Two sites in south Florida, each with abundant water lettuce but differing in <i>Mansonia</i> densities, were chosen for comparisons by regular sampling of the plant and invertebrate communities in accompaniment with abiotic measures which included water chemistry. All life stages of <i>Mansonia</i> were more abundant at Chinese Farm (CF) than at Highway 614 (HWY 614), and a great discrepancy in larval abundances at the two sites suggested that larval mortality was higher at HWY 614. Freezing temperatures caused greater hibernial mortality of <i>Pistia stratiotes</i> and reduced root biomass more severely at HWY 614 than at CF. Water lettuce regrowth in the spring was manifested by production of many small leaves, and biomasses and leaf areas reached steady-state maxima in the fall and early winter, which coincided with periods of peak <i>Mansonia</i> abundance. Among water quality measures, only pH and total phosphates differed significantly between sites, and no limiting nutrients were identified.					
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Diptera dominated numerically among insects identified from emergence traps placed over water lettuce and among aquatic invertebrates collected from P. stratiotes roots. However, Odonata accounted for over 75% of the biomass of insects recovered from aquatic samples. Exoskeletal remains in guts of both Odonata and the mosquitofish Gambusia affinis confirmed these as natural predators of Mansonia larvae and pupae. Tests in aquaria documented G. affinis as an effective biocontrol agent of Mansonia.

Egg masses of Mansonia were recovered on both the upper and under surfaces of P. stratiotes leaves. Of the two Mansonia inhabiting water lettuce in Florida, only M. dyari is capable of upper-surface oviposition, this behavior regarded as an evolutionary specialization to the growth form of water lettuce. Masses laid above water line on middle-aged leaves approached submersal as leaves senesced during the long period of egg incubation. Egg masses were highly clumped in nature and oviposition site selection was influenced by leaf angle from the horizontal.

Some interplant movement of Mansonia was confirmed by recruitment of larvae to 'cleaned' roots. However, nocturnal dispersal of larvae was not observed.



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SUMMARY

The current report describes the results of 2.5 years of study of factors regulating populations of Mansonia dyari, a pest mosquito and potential disease vector in south Florida. Sites of high and low M. dyari density were selected, in northern St. Lucie County and through regular sampling compared in particular characteristics which included: abundances of all mosquito life stages, seasonal growth of the host plant Pistia stratiotes, water quality, and associated invertebrate fauna. (KT)

Fortnightly surveys for 22 months revealed higher densities of Mansonia egg masses at an abandoned aquaculture pond (CF) than at a roadside drainage ditch (HWY 614). Egg densities peaked in the autumn. Mansonia larvae and pupae recovered from P. stratiotes in monthly quadrat samples were on average 10X more abundant at CF than HWY 614. Larvae peaked in density in the autumn and early winter. Adult Mansonia captured in emergence traps placed over water lettuce were also more abundant at CF on most sampling dates. Mansonia titillans was found to represent a significantly higher proportion of the total Mansonia crop at HWY 614 compared to CF.

Measurements of P. stratiotes growth revealed comparable seasonal patterns at both sites: winter mortality of larger plants, spring recrudescence by vegetative budding, and achievement of maximum leaf areas and plant biomass in autumn and early winter. Hibernial mortality of water lettuce was more severe at HWY 614, and root biomasses approached zero for several sampling occasions at this site after winter cold. Peak flowering occurred synchronously at both sites in December. The growth and declination of individual P. stratiotes leaves were measured and expressed as functions of leaf age.

Nitrates, nitrites, phosphates, ammonium, dissolved oxygen, pH and water temperatures were measured monthly on water samples from both sites. Concentrations of total phosphates were dramatically higher at CF and the water pH slightly more acidic at HWY 614.

Invertebrate fauna captured in emergence traps over water lettuce and the aquatic invertebrates associated with P. stratiotes roots were counted and classified, and all identifications resolved to date are provided as appendices to this report. Diptera numerically dominated in both types of collections, but Odonata were estimated to account for over 75% of the biomass of invertebrates on P. stratiotes roots. Seven species of odonates were recognized, and monthly length and density measurements allowed growth and survival estimates of the most abundant species.

Mansonia egg masses were recovered on both the upper and under surfaces of P. stratiotes leaves. Mansonia titillans was shown to be capable only of under-surface oviposition typical of members of this genus, but M. dyari was labile in egg placement

behavior. Approximately two-thirds of M. dyari egg masses were on upper leaf surfaces of intact plants, selection of these sites regarded as an evolutionary specialization to the growth form of P. stratiotes. Upper-surface egg masses laid out of water approach submersal during the long period of incubation while senescing leaves droop towards the water surface. When gravid M. dyari were offered flat leaves, all eggs were deposited on under surfaces. Among all leaves surveyed, the distribution of egg masses was highly clumped. The mean angles of leaves with eggs were no different for upper and under-surface masses.

Predation upon Mansonia larvae in nature was confirmed by gut content analyses of mosquitofish, Gambusia affinis, and common odonates. Among captured predators with identifiable invertebrate remains in guts, 43.5% of G. affinis and 20.7% of odonates contained pieces of Mansonia exoskeleton. The ability of G. affinis to glean Mansonia from roots was confirmed in experiments with larvae attached to P. stratiotes in aquaria.

Day and night sampling at CF did not support the published notion of nocturnal larval dispersal of Mansonia. Some interplant movement by mature larvae was confirmed by experiments which assessed recruitment to 'cleaned' P. stratiotes set in ponds. The stimuli responsible for larval dispersal remain unresolved, but may be related to larval density.

## FOREWORD

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## I. Statement of Problems

In southern Florida Mansonia dyari is a pest mosquito and potential vector of St. Louis encephalitis (1). This species is closely associated with water lettuce, a tropicopolitan floating macrophyte common in freshwater lakes, ponds and canals (2). The attachment of larvae and pupae to the roots of aquatic plants protects Mansonia mosquitoes from conventional mosquito control methods. Owing to difficulties in sampling and rearing, the ecology and behavior of M. dyari, especially its interrelationship to its host plant P. stratiotes and co-occurring aquatic organisms, have been poorly studied.

## II. Background

Mosquitoes of the genus Mansonia (s.s.) are vectors of important tropical diseases such as filariasis (3,4), encephalitis (5), and other viral maladies afflicting man (6). Without exception, members of the genus attach by larval siphons or pupal trumpets to the root systems of aquatic plants. Earlier field studies on this mosquito group in Florida described sampling methods and seasonality (7-10) and distribution among available plants (8,11). Slaff and Haefner (11) confirmed that M. dyari preferred water lettuce for larval attachment.

Neither in Florida nor elsewhere have there been concerted attempts to investigate factors which regulate natural populations of Mansonia. Any effort to investigate Mansonia life histories must incorporate information about the distribution and abundance of its host plant. Surprisingly, because of its abundance in Florida, the growth and productivity of P. stratiotes has been examined only by Odum (12) in a spring habitat atypical for this macrophyte. No studies examining biotic interactions between Mansonia and co-occurring aquatic organisms have been undertaken in Florida save for preliminary observations in aquaria (13).

## III. Rationale

The research described herein was undertaken in the expectation that appropriate observations and experiments would reveal interrelationships between the abundance of M. dyari, the growth of its host plant, water quality, associated aquatic organisms, and specialized behaviors adaptive to the P. stratiotes host. Because of the diversity in biological systems and insights gained by comparisons, two field sites, both containing abundant water lettuce but known to differ in M. dyari densities, were contrasted in most measures of the plant and invertebrate communities.

#### IV. Experimental Methods

##### Site description

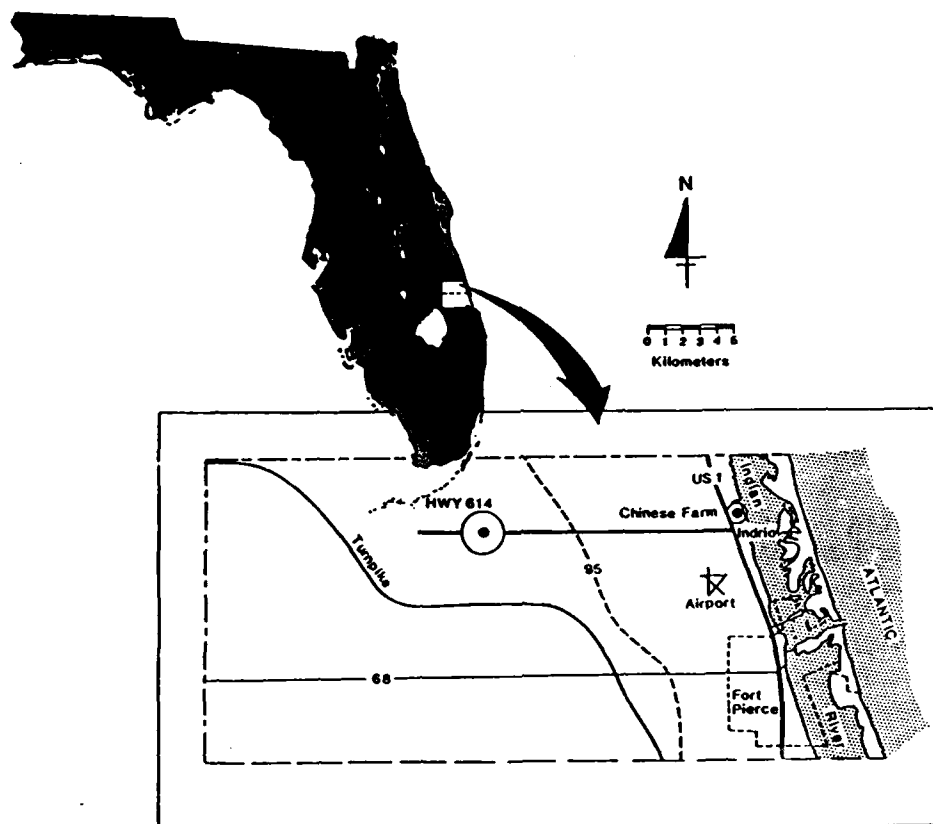


Fig. 1. Location of study areas in northern St. Lucie County, Florida. Chinese Farm (CF) was composed of abandoned aquaculture ponds and Highway 614 (HWY614) a drainage canal along a roadside.

The two field sites (ca 27°30' N, 80°30' W) were located on the eastern coast of Florida in northern St. Lucie County (Fig.1). This region is characterized by a subtropical climate with seasonal rainfall occurring primarily in the summer and autumn (Figs. 2, 3). The Chinese Farm (CF) site was composed of abandoned aquaculture ponds (17 X 10 m) colonized by P. stratiotes. One of these ponds (A8), its perimeter vegetation consisting mostly of high grass, was used for all regular plant and invertebrate trapping between September 1985 and June 1987. The Highway 614 site, a roadside drainage canal 9 m in width containing P. stratiotes, was monitored for the same time period. Water lettuce grew in monocultures at both study sites.

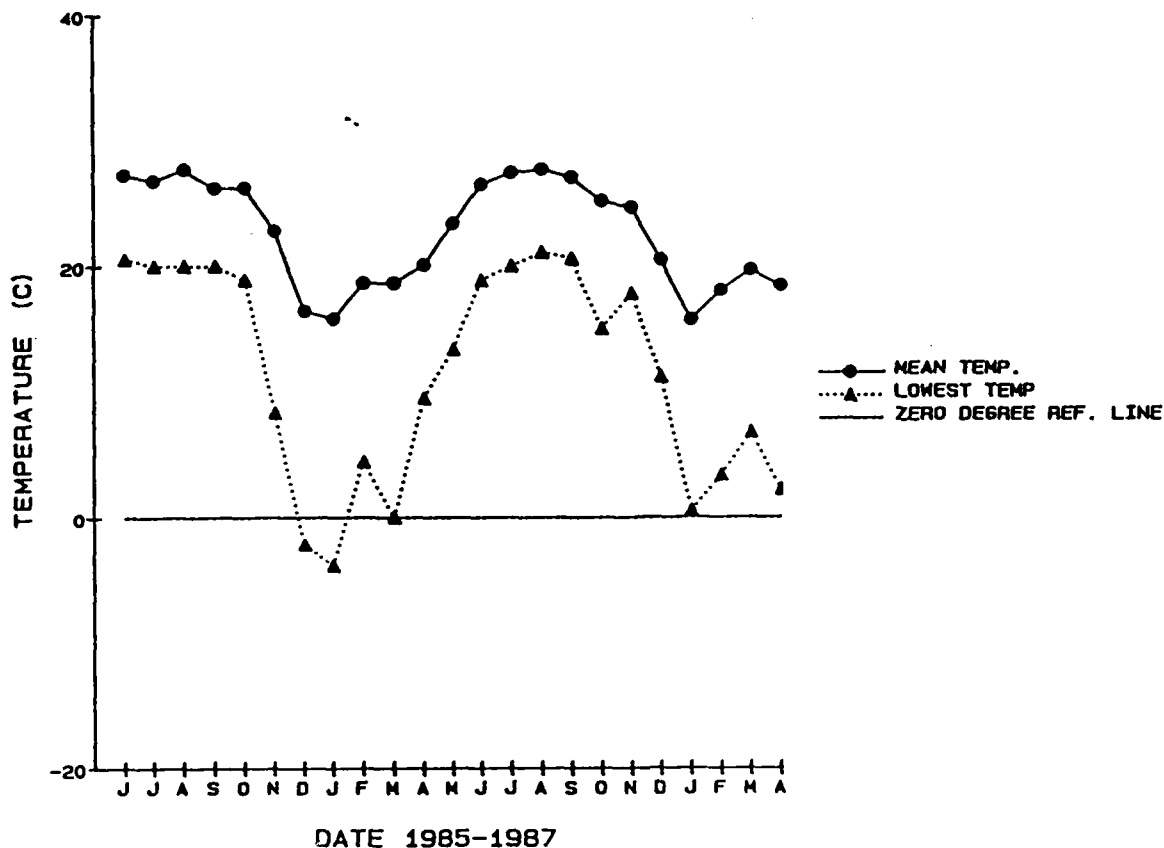


Fig. 2. Monthly low and mean ambient temperatures during the study period at a site 10 km to the northwest of CF and HWY614.

### Growth of water lettuce

The methods used for sampling plants were a modification of those described by Hall & Okali (14) for measuring growth of P. stratiotes on a tropical lake in Ghana. Rectangular grids 2 X 5 m were established at CF and HWY614, their perimeters defined by PVC pipes. Each grid was considered to consist of 96 discrete quadrats each measuring 30 X 30 cm, after exclusion of a 10 cm border around the interior of the grid. At approximately 30 day intervals, five quadrat samples were selected at random, excluding any quadrats contiguous to one another. No samples were collected during February 1986 due to a temporary scarcity of P. stratiotes.

Each quadrat sample consisted of a 900 cm<sup>2</sup> section of vegetation and the underlying water column to an average depth of 35 cm. Quadrat samples were collected using a stainless steel sampling tool 30 X 30 X 70 cm with serrated teeth around the perimeter of the bottom to penetrate the P. stratiotes mat (Fig.4). A trap door inside the tool was closed after penetration of the vegetation allowing the underlying water column to be collected with the sample.

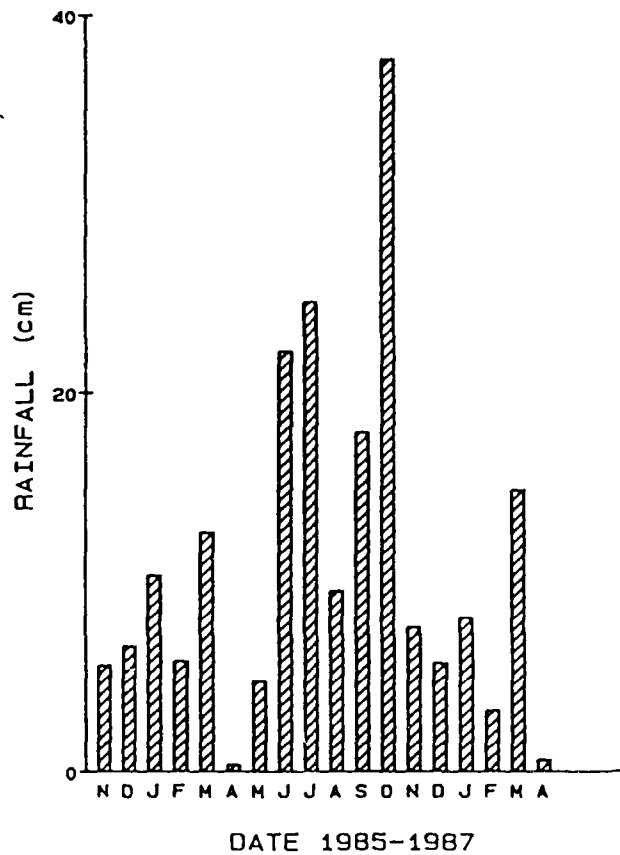


Fig. 3. Monthly rainfall accumulations from the weather station 10 km northwest of the study sites.

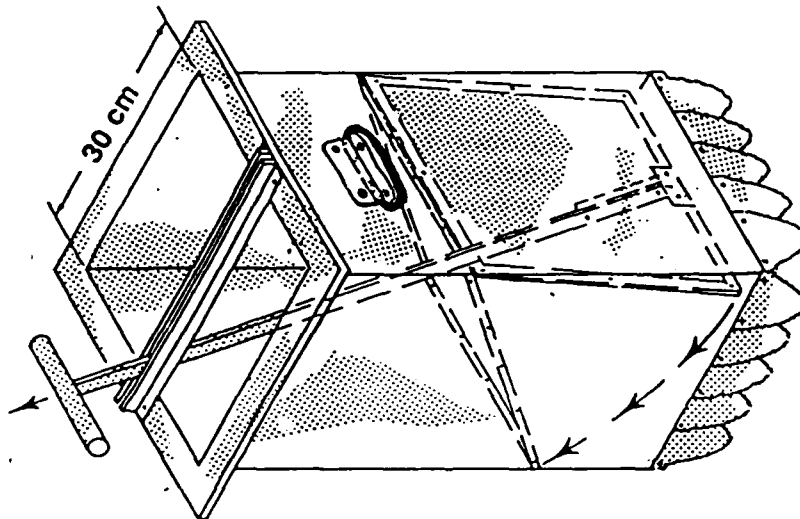


Fig. 4. Sampling tool used for cutting and removing 900 cm<sup>2</sup> quadrats of water lettuce and associated fauna.

Each sample was placed in a 20-litre bucket and returned to the laboratory. The number of P. stratiotes plants were counted as well as the number of leaves and flowers on each plant. Since some plants and leaves were cut by the sampling tool, fragments consisting of 50% or greater of a plant rosette were counted as a whole plant, while lesser portions of rosettes were not tallied. Complete or partial leaves that were attached to a rosette were included in counts of the number of leaves per plant. Both attached and detached leaves were included in measurements of leaf surface area determined with a Licor Leaf Area Meter. Dry weights of leaves and roots were measured after oven drying at 80° C for 48 hours. Leaves that were more than 50 % decayed were not included in any of the above measurements.

Relative growth rates (RGRs) of P. stratiotes were estimated following the methods of Hall & Okali (14) except that leaf and root, but not rhizome, biomass were used. Hall & Okali reported that changes in biomass did not significantly affect the ratio of leaf to non-leaf components, the leaves accounting for approximately 90% of the plant biomass in their study. Therefore, RGRs calculated on the basis of leaf and root biomass should be comparable to RGRs calculated on the basis of whole plant biomass, although the more severe winters of subtropical Florida compared to tropical Ghana might effect significant changes in the ratios of plant components. The formulae of Hughes & Freeman (15) were adapted for RGR calculations:

$$RGR = 1/W \times dW/dt$$

$$\text{approximated by: } RGR = 1/W_0 \times (W_1 - W_0)/(t_1 - t_0)$$

where W = observed biomass

and  $W_n$  = observed biomass at time  $t_n$

At CF and in plastic wading pools at the Florida Medical Entomology Laboratory (FMEL) 6.6 km to the north of the study sites, the growth rates of individual P. stratiotes plants were measured by recording changes in lengths of individual leaves and changes in leaf angles from the horizontal with time. Individual leaves were tagged as they unfurled (day 0) and lengths and angles recorded twice weekly, these measurements terminating either when an entire leaf contacted the water surface and subsided, or was badly damaged by insect grazing. These observations were made from late July through early October 1986 when leaf surface area was high (Figs. 4 & 5) and neighboring plants were closely packed in nature.

#### Water chemistry

Monthly water samples from the two sites were analyzed with Hach Kit reagents. Dissolved oxygen (D.O.) was determined by the modified Winkler titration method, ammonium nitrogen by

Nesslerization, nitrate-nitrogen by diazotization, and total phosphate by the ascorbic acid method. An Orion pH meter (model 211) was used to measure pH in situ at the time of water collection (early PM) when water temperatures were also taken.

#### Monitoring *Mansonia* life stages

From September 1985 through June 1987, fortnightly egg mass surveys were made at CF and HWY 614. Both the upper and under surfaces of 100 or more leaves, > 10 cm in length, were inspected on ten or more plants for unhatched *Mansonia* egg masses. Plants were selected haphazardly by two or more inspectors working different parts of the habitat. Collected egg masses were brought to the laboratory to confirm viability by hatching. First instar larvae of the first 150 collected egg masses were preserved in 70% ethanol and the head capsule widths of 25 larvae from each mass were measured under 50X to separate *M. dyari* from *M. titillans* according to the size criteria reported by Nemjo & Slaff (16).

Larvae and pupae of *Mansonia* were dislodged from *Pistia* roots by vigorous shaking of plants collected in monthly quadrat samples. Live invertebrates were sorted from debris in enamel pans after washing the non-vegetative portion of samples with clean water in sieves. *Mansonia* larvae were counted and separated by instar, but *M. dyari* were not discriminated from *M. titillans*.

The weekly emergence of adult *Mansonia* was monitored at each site on alternate weeks with two pyramidal traps which floated atop one square meter of *P. stratiotes* (17). Insects caught upon the adhesive Tack Trap plate at the apex of the pyramid were brought to the laboratory for preliminary identifications, including the separation of adult *M. dyari* from *M. titillans*. The sticky plates used for trapping were kept off of pyramids on alternate weeks. Before plates were reinstalled, traps were cleaned of spiders and aerial predators, esp. adult Odonata. At six to eight-week intervals, trap locations were changed to allow for possible local depletion of *Mansonia*.

#### Oviposition site selection

Preliminary surveys for *Mansonia* eggs revealed the location of masses on both the under and upper surfaces of *P. stratiotes* leaves. Since members of this genus were not previously known to lay eggs on the upper surfaces of floating macrophytes, oviposition site selection was examined in greater detail. Leaves with viable masses collected at CF and HWY614 between September 1985 and April 1986 were brought to the laboratory where egg mass locations were mapped in relation to the leaf edge, topography, and a 'water line' 3-8 cm from the leaf base. For all surveys, the number of egg masses on the upper versus under surfaces of leaves was recorded. The number of egg masses on a leaf and on a plant were scored to obtain evidence of clumping (overdispersion)

of masses.

For oviposition site selection experiments in the laboratory, inseminated Mansonia females were captured in 'lard-can' traps (18) baited with a chicken. Females were allowed to blood-feed on a chicken, then held for 7-8 days at 24-26°C in a cage (900 square cm) without access to an oviposition site. For experiments, oviposition substrates were inserted overnight in a tray of water, and the locations of egg masses scored on the following day.

For egg masses of known ages, the timing from oviposition to hatch was measured in the laboratory at 20 and 26 ° C. To investigate a possible relationship between egg mass age and leaf angle from the horizontal in nature, angles of leaves with viable masses were measured at CF, and eggs were returned to the laboratory and incubated to determine the date of hatching at a constant temperature.

Because many viable egg masses were found out of water, experiments were conducted to determine the survivorship of larvae which hatch before eggs are submersed. Egg masses were incubated on damp filter paper until the day of hatch, this event recognized by the lifting of the micropylar egg cap by first instar larvae. Egg masses were submersed one, two or three days after hatching, and the number of viable and dead larvae recorded.

#### Other fauna

Attempts to identify all fauna in quadrat samples are still in progress at this writing. Collected specimens were morphotyped and sent to specialists for identifications. Of these, generic and specific names were obtained for certain groups such as Odonata and certain Coleoptera, but many other invertebrates remain identified only to family level (Appendix I).

Except for the minute Crustacea and Acarina, numbers of individuals of each morphotype were recorded for each quadrat collection. The length of every odonate specimen was measured. For other groups, the average lengths of individuals were estimated from preserved aliquots of each morphotype. The length measurements were used to estimate the biomass contributions of the respective insect orders according to regression equations provided by Smock (19).

Adult insects caught in emergence traps were also sent to taxonomists for identifications. Genus and species names acquired to date are listed in Appendix II.

#### Predation on Mansonia larvae

Preliminary experiments revealed that potential predators, notably fish and odonate larvae, retained recognizable pieces of exoskeleton in their guts after ingestion of Mansonia larvae. On four occasions odonate larvae were dislodged from Pistia roots at

CF and preserved in 70% ethanol within one hour after capture. Larval lengths were measured and, in some instances, predators were identified to species before gut dissections. Guts were scored either as empty (without food), with unidentifiable remains, or remains of identified invertebrates, including Mansonia larvae which were recognized by their saw-toothed siphons.

On two occasions fish were captured with a Hart trap (20) or 20-litre bucket and immediately preserved in fixative. Fish were identified to species, measured, and dissected to examine invertebrate remains in their guts. At the conclusion of these observations, 17 aquaculture ponds which contained P. stratiotes were surveyed for fish with Hart traps and for Mansonia larvae and pupae by shaking three plants per pond.

To investigate further fish predation on Mansonia, known numbers of Gambusia affinis or Heterandria formosa were introduced into aquaria which contained P. stratiotes and known numbers of attached larvae. After 48 hours of exposure, the contents of the aquaria were examined and compared to control tanks which had no introduced predators. Similar laboratory experiments were performed using odonate larvae captured in quadrat samples as predators of Mansonia larvae attached to P. stratiotes in beakers or aquaria.

#### Larval dispersal

Bailey (21) reported that nocturnal collections of Mansonia from water lettuce yielded significantly fewer larvae than diurnal collections. On the basis of these results he hypothesized that larvae detach and disperse among plants at night. To substantiate the nocturnal/diurnal difference in Mansonia abundance on water lettuce roots, Bailey's (21) sampling regimen was repeated in a pond at CF. Ten plants were chosen haphazardly at 9:00 PM and their larval and pupal numbers compared to the yields from ten plants collected from the same pond at 9:00 AM the following morning.

A separate set of experiments examined the colonization of 'cleaned' P. stratiotes plants deposited in ponds with known Mansonia densities. Ten to twenty large plants were brought to the laboratory and vigorously shaken in 20-litre buckets to dislodge all larvae and pupae. The numbers of larvae and pupae recovered from five plants were recorded, and cleaned plants were carefully returned to their original habitats. At one hour and one, four and eight days after setting, five of the cleaned plants were retrieved and the numbers and instars of attached larvae identified in the laboratory by previously described methods.



## V. Results

### Seasonal growth of water lettuce

Both average leaf size and leaf area per plant declined in the winter and did not increase again until late spring in 1986 (Figs. 5 & 6). At both sites a similar pattern was observed in 1987, but the decreases were less, likely because of the absence of sub-freezing temperatures in the winter of 1987 (Fig. 2). Owing to hibernal mortality of larger plants and the vegetative propagation of small immatures, plant and leaf density peaked late in the winter of 1986 at CF (Figs. 7 & 8). Because the winter depression of *P. stratiotes* persisted longer at HWY 614 (Figs. 5 & 6), the post-winter peak in leaf and plant density was delayed until early summer at this site (Figs. 7 & 8).

Plant density was inversely related to plant size and appeared to fit negative exponential relationships at both CF and HWY 614 (Fig. 9). Maximum plant densities and leaf areas were 2-4X higher at CF than at HWY 614.

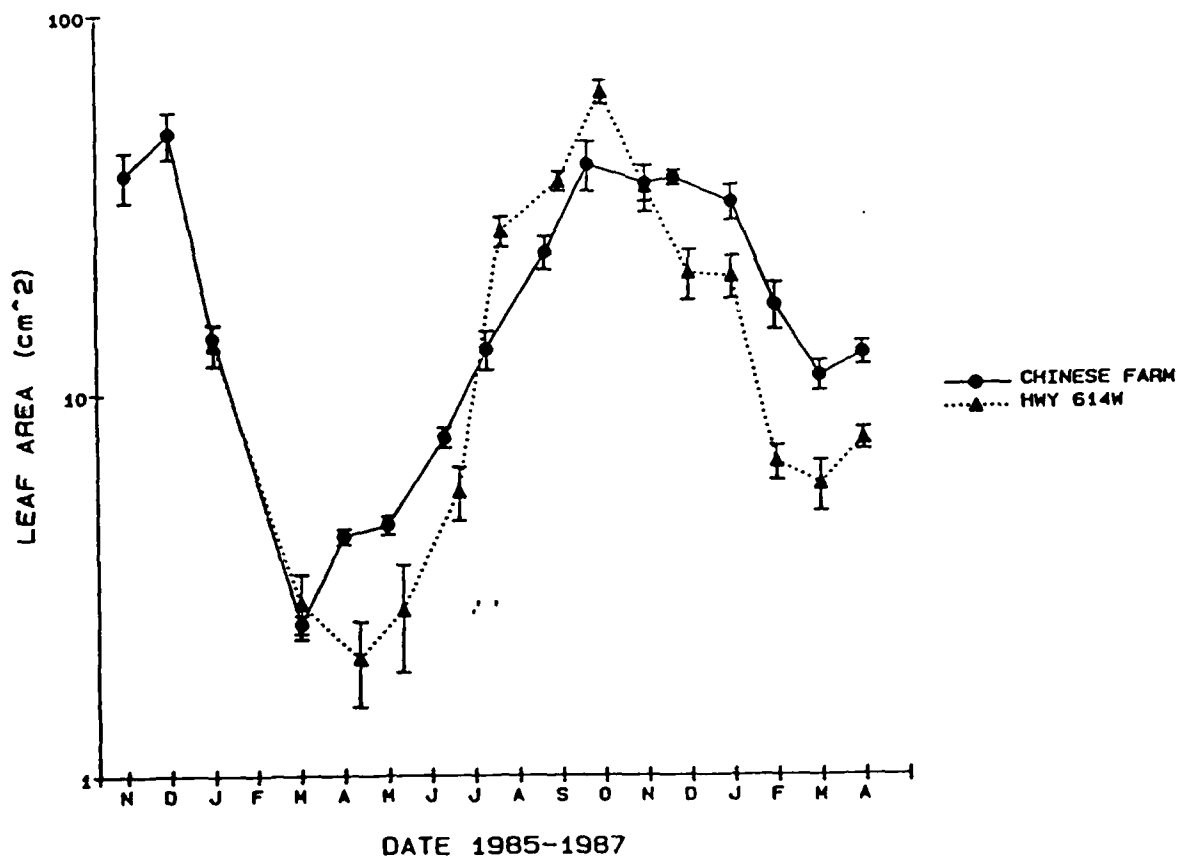


Fig. 5. Seasonal changes in mean area per leaf of *P. stratiotes* from five quadrat samples collected monthly at two sites. Vertical lines denote  $\pm 1$  s.e.

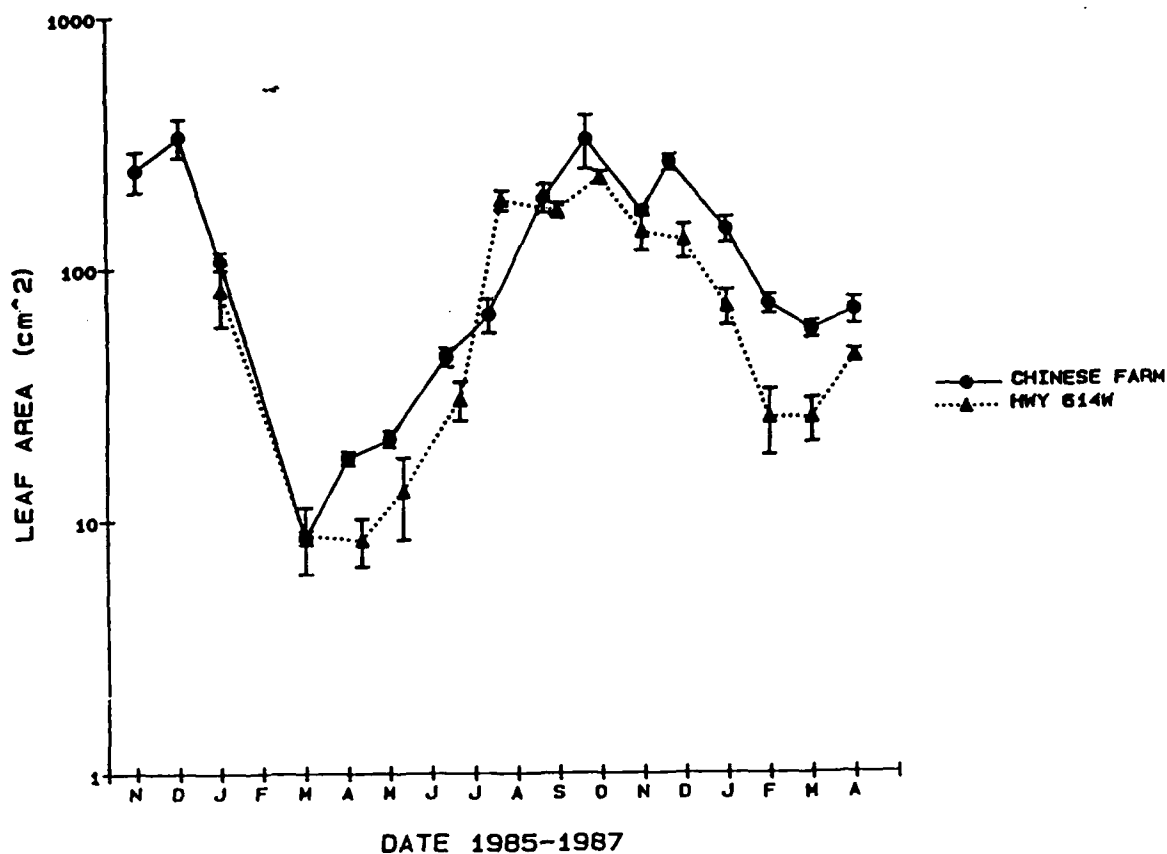


Fig. 6. Seasonal changes in mean leaf area per plant of *P. stratiotes* from five quadrat samples collected monthly at two sites. Vertical lines denote  $\pm 1$  s.e.

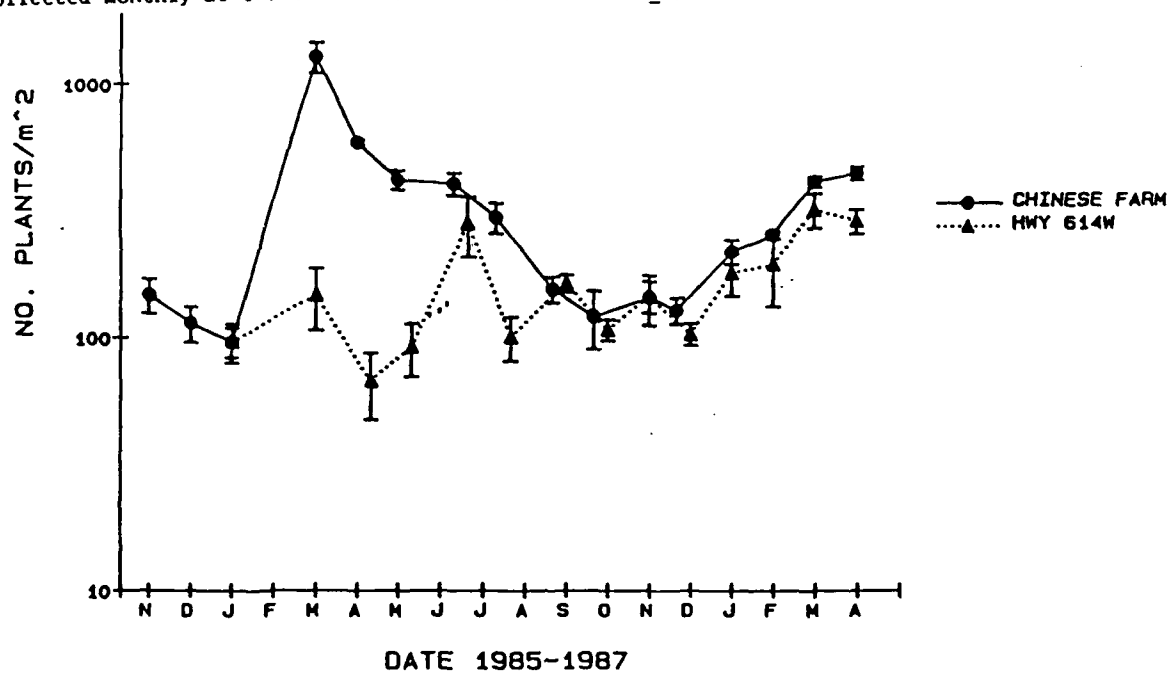


Fig. 7. Monthly plant densities of *P. stratiotes* in five quadrat samples collected at two sites. Vertical lines denote  $\pm 1$  s.e.

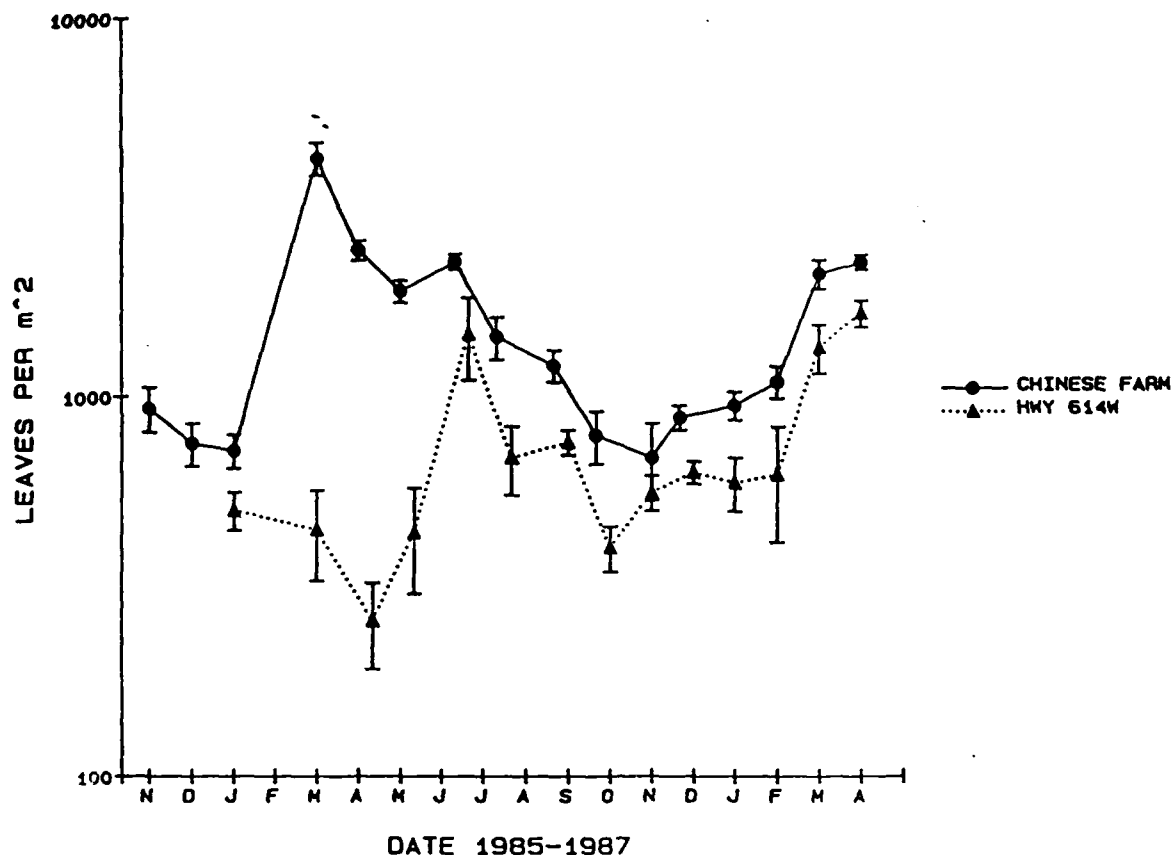


Fig. 8. Monthly leaf densities of *P. stratiotes* in five quadrat samples collected at two sites. Vertical lines denote  $\pm 1$  s.e.

A pronounced seasonality was observed in the time of flowering, quadrat samples taken in December of 1985 and 1986 exhibiting more than twice the number of flowers per plant than observed at any other time of the observation period (Fig. 10). Several immature seeds were recovered from sampled plants in December 1986, but none at any other time.

At most sampling periods average root biomass was approximately ten times greater at CF than at HWY 614 (Fig. 11). The winter mortality of *P. stratiotes* at HWY 614 reduced root availability to near zero from March through May of 1986. The composite biomass of roots plus leaves was less at HWY 614 than at CF during all sampling intervals but one (Fig. 12).

RGRs observed at CF fluctuated between 0.2 and -0.2 g/g/week (Fig. 13), this range similar to that observed by Hall & Okali (14) for *P. stratiotes* in Ghana. Negative growth rates occurred primarily in the winter months at both CF and HWY 614. RGR values well above 0.2 g/g/week occurred at HWY 614 after the winter die-off of *P. stratiotes* when plant biomass had dropped to near zero at that site.

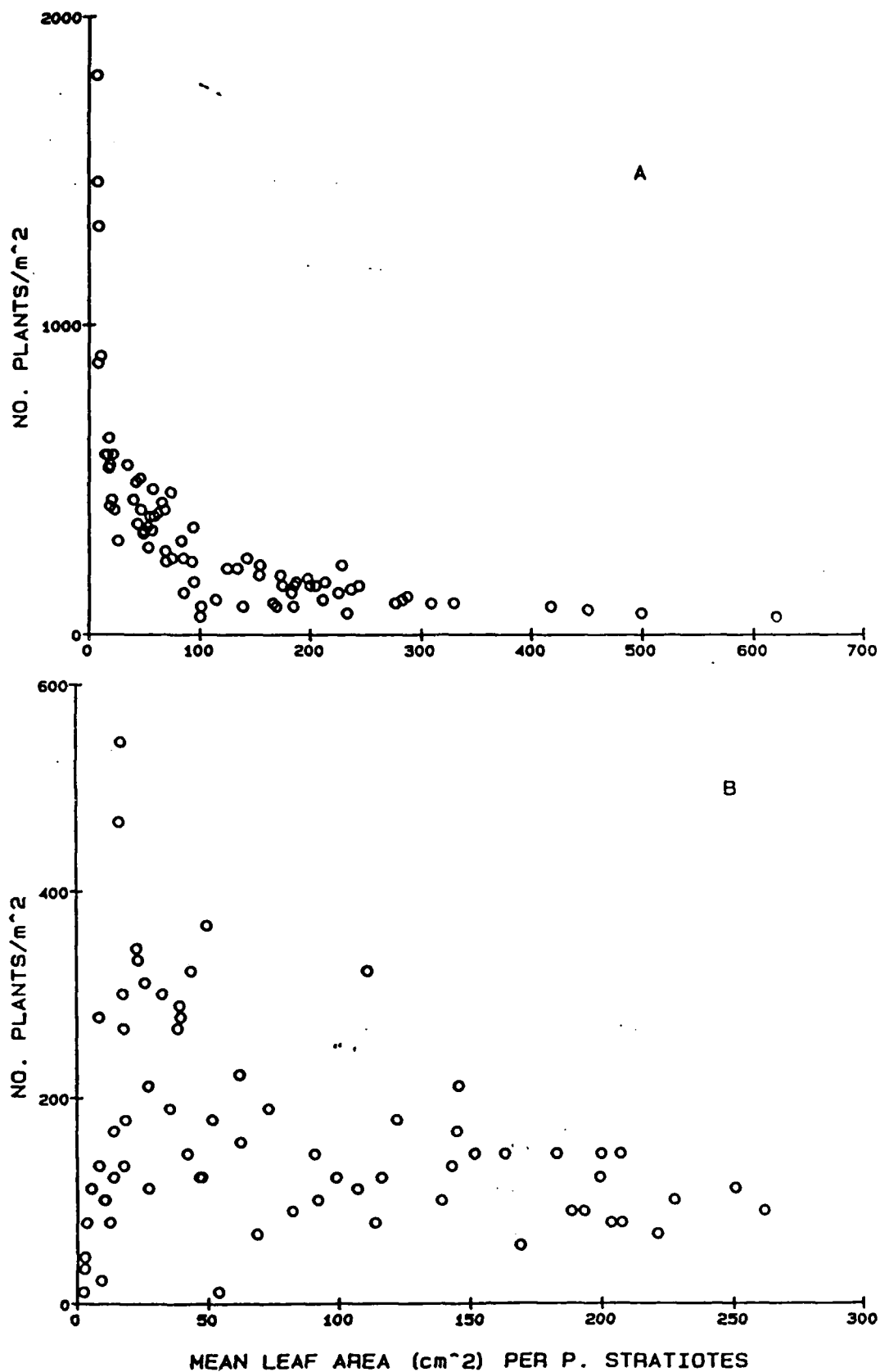


Fig. 9. Water lettuce plant density plotted versus mean leaf area per plant from quadrat samples collected monthly from November 1985 through April 1987 at (A) Chinese Farm and (B) Highway 614.

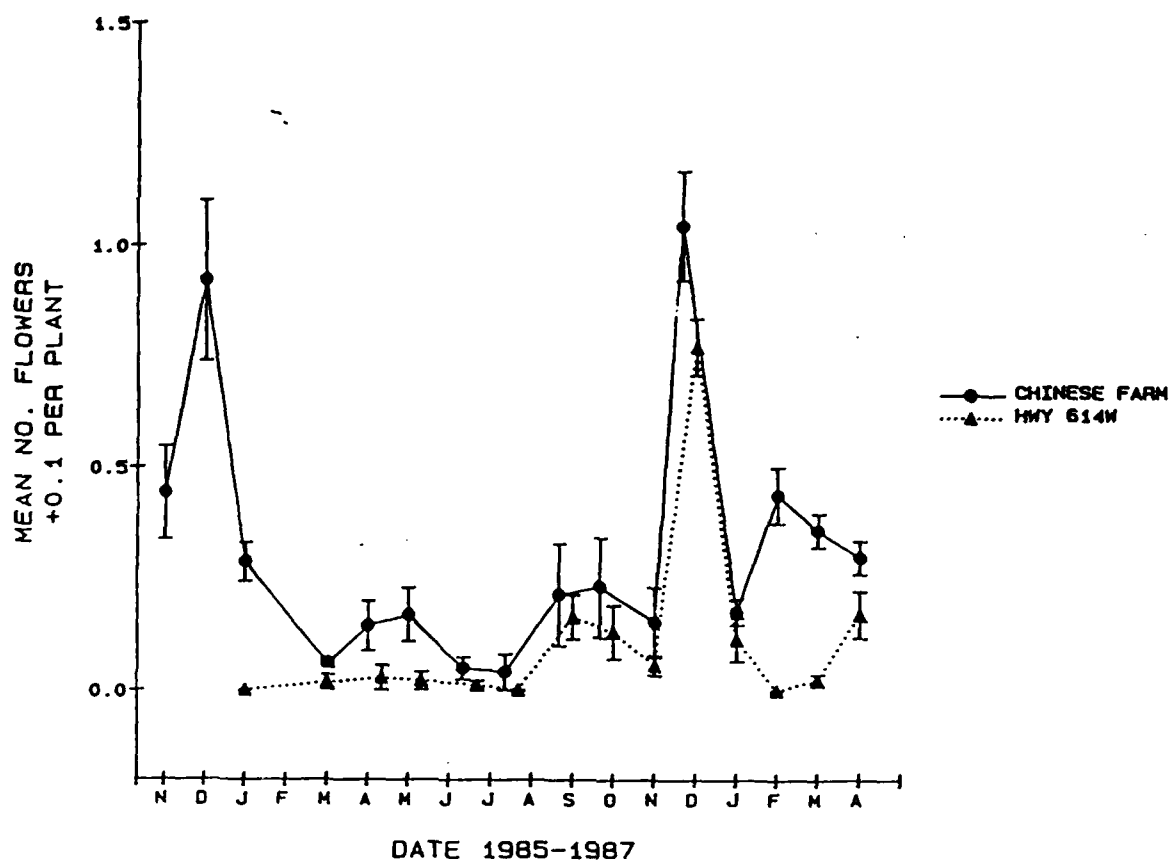


Fig. 10. Monthly flower densities of *P. stratiotes* in five quadrat samples collected at two sites. Vertical lines denote  $\pm 1$  s.e.

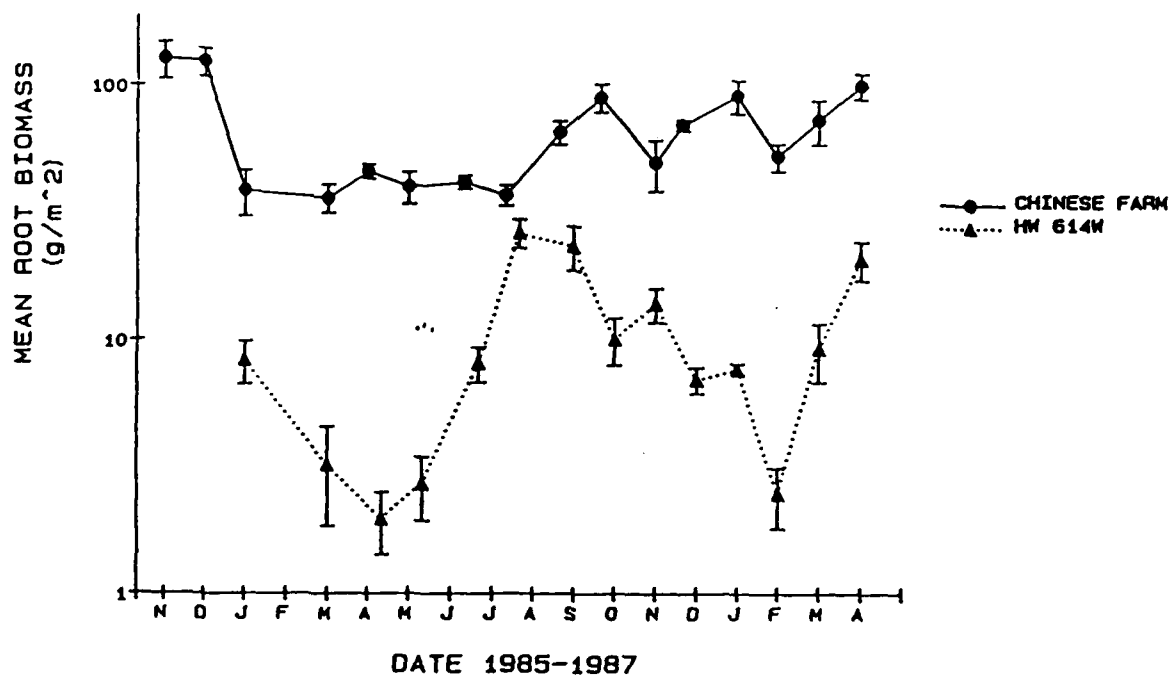


Fig. 11. Monthly root dry weights of *P. stratiotes* from five quadrat samples collected at two sites. Vertical lines denote  $\pm 1$  s.e.

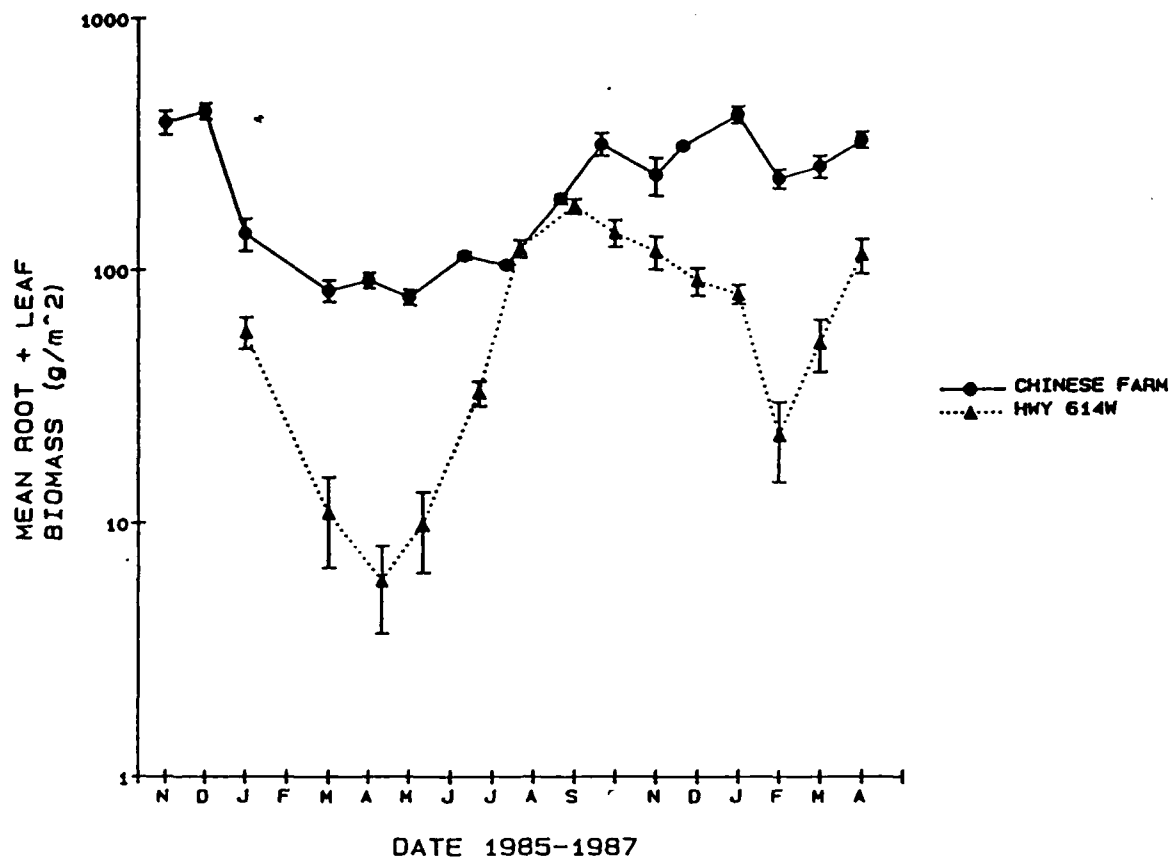


Fig. 12. Monthly root plus leaf dry weight from five quadrat samples collected at two sites. Vertical lines denote  $\pm 1$  s.e.

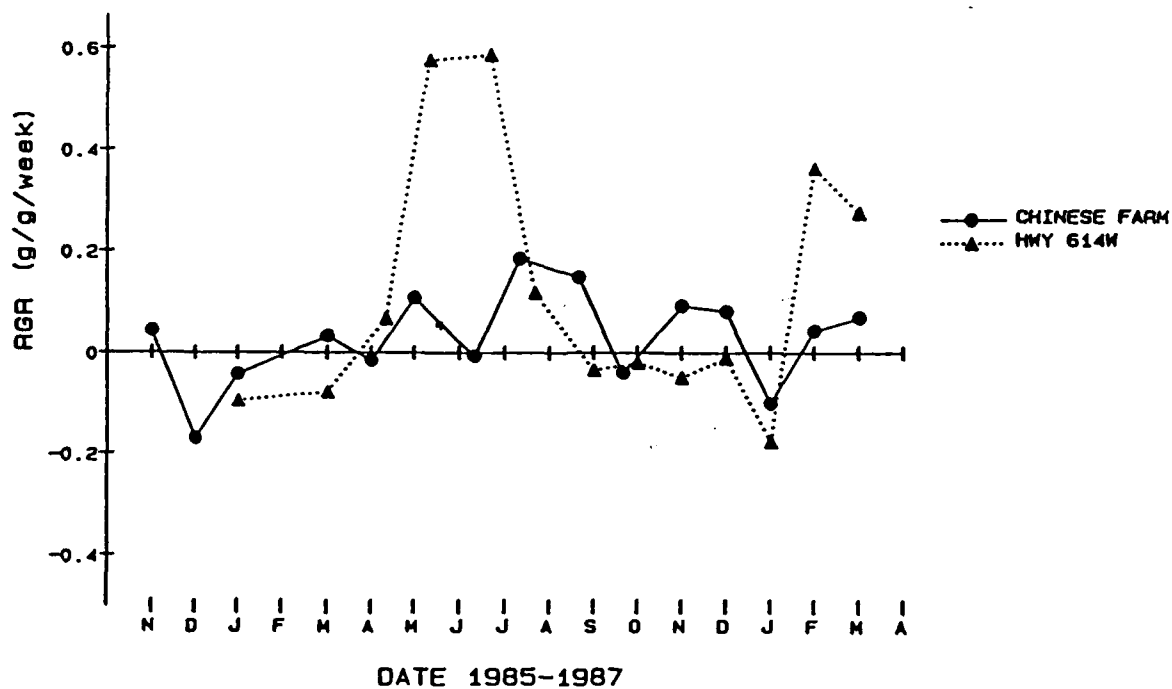


Fig. 13. Changes in Relative Growth Rate (RGR) based on monthly biomass determinations of *P. stratiotes* from quadrat samples.

The increase in leaf length was fastest during the first week after unfurling, the rate of elongation declining in successive weeks thereafter (Fig. 14). Leaves in the wading pool at FMEL did not grow as large as those at CF during the 30-day observation period. The average angle of leaves with respect to the water surface declined with leaf age at similar rates at both CF and FMEL (Fig. 15). Plants at CF were more densely packed than at FMEL, the pressure of neighboring plants at the first site maintaining higher angles with respect to the horizontal.

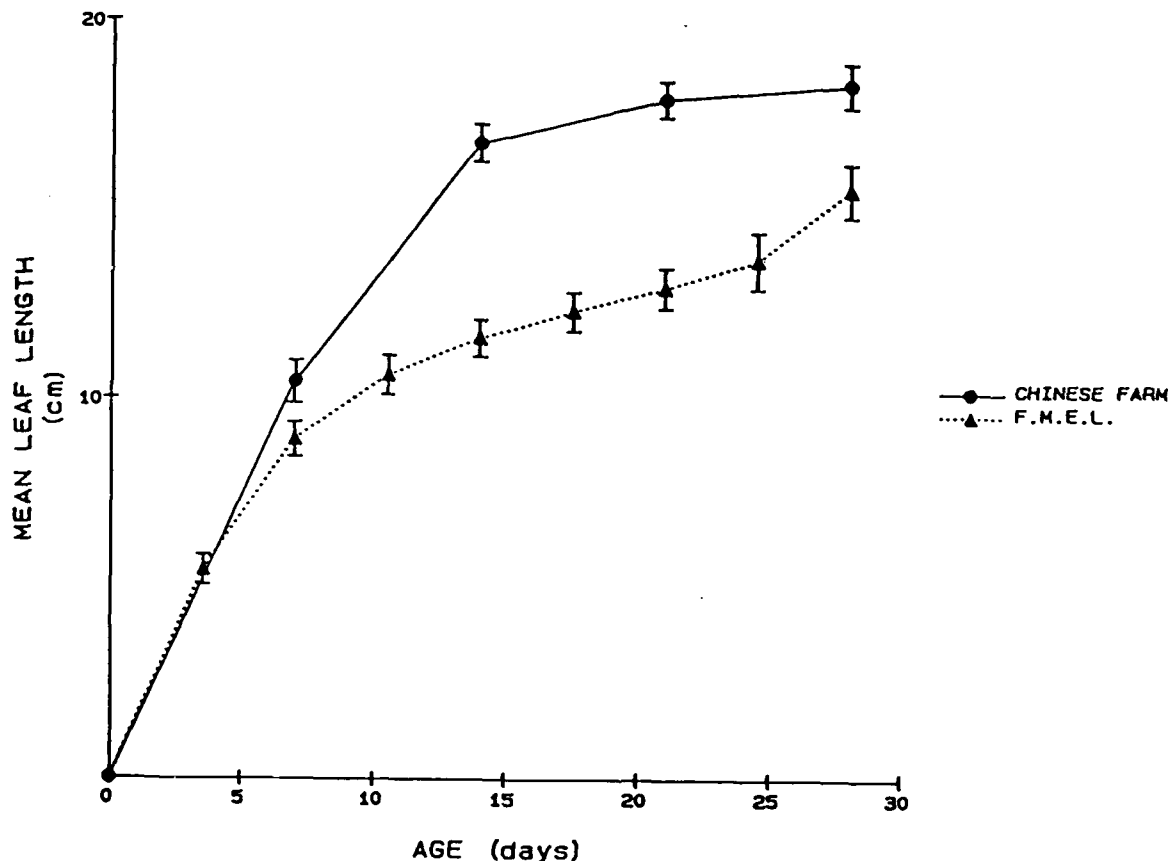


Fig. 14. Growth of *P. stratiotes* as indicated by changes in leaf length from the day of unfurling (day 0). FMEL plants (n = 25 leaves measured) grew in a plastic wading pool with pond water from CF. Chinese Farm plants (n = 38 leaves) were measured in undisturbed ponds.

#### Water quality

On all sample dates nitrates and nitrites were below the limits of detection (0.033 and 0.088 mg/ml, respectively) of the Hach Kit. Ammoniacal nitrogen and dissolved oxygen were slightly higher at CF than at HWY 614, but the differences were not significant (Table I). However, total phosphates were more than 40X higher at CF than HWY 614, this difference highly significant. The pH at HWY 614 was slightly acidic and consistently less than the neutral pH at CF. Daytime (PM) temperatures recorded at the two sites were not statistically different.

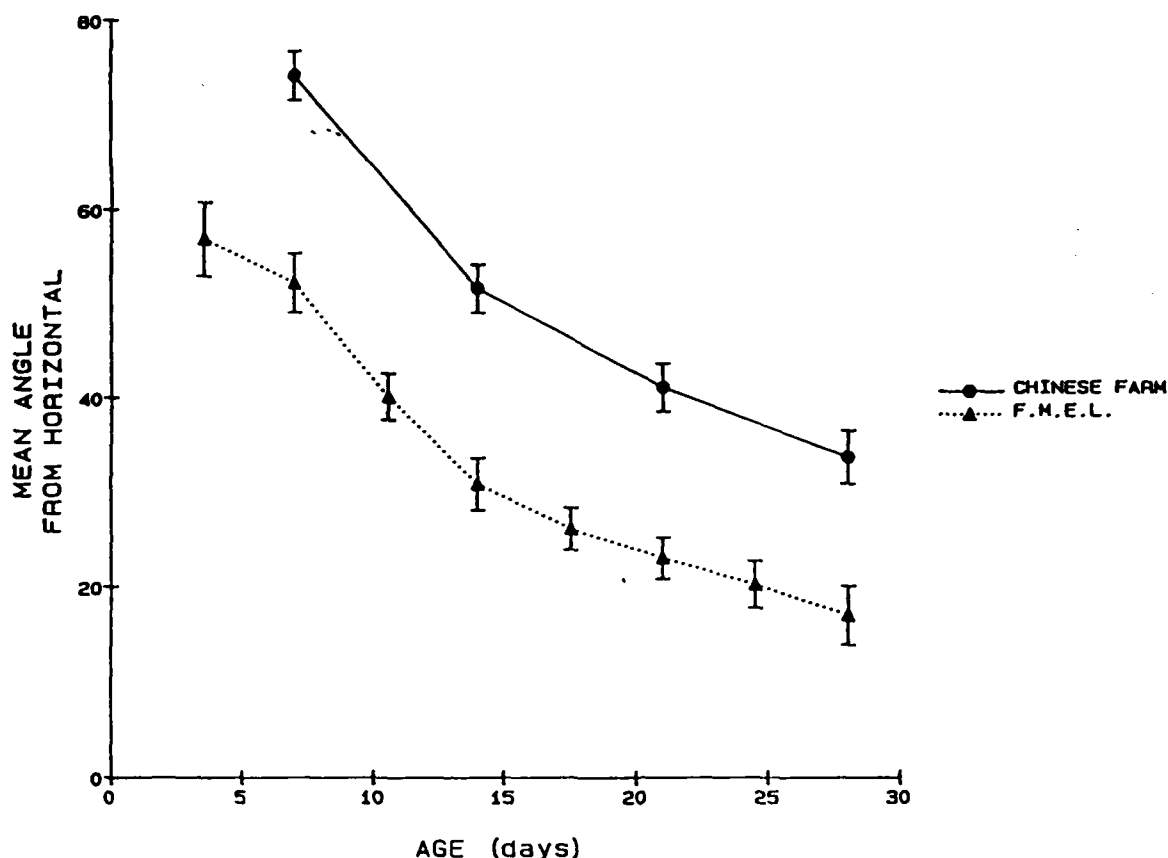


Fig. 15. Changes in leaf angle with respect to the water surface as a function of leaf age of *P. stratiotes*; n = 43 leaves for CF, n = 27 leaves for FMEL.

Table I. Mean values ( $\pm 1$  s. dev.) of water quality measurements taken monthly from October 1985 to April 1987.

	$\frac{+}{-}$ NH 4 (mg/ml)	$\frac{-3}{+3}$ PO 4 (mg/ml)	D.O. (mg/ml)	pH	water temp. (C)
n	15	17	15	14	17
CF	1.54(0.47)	20.88(2.93)	2.14(1.62)	7.01(0.25)	20.53(3.57)
HWY614	1.23(0.46)	0.51(0.40)	1.21(1.53)	6.74(0.30)	19.93(3.33)
t*	1.89	28.37	1.61	2.60	0.62
P	ns	<0.001	ns	<0.05	ns

\* = unpaired t-test, variances not assumed equal

### Mansonia life stages

Egg mass density was lowest between January and June and highest during the latter half of each year (Fig. 16). The average number of masses per leaf was higher at CF than HWY 614 on most sampling dates, a collection in late August 1986 being a notable exception. Egg mass densities over the entire observation period averaged 0.050 and 0.034 masses per leaf at CF and HWY



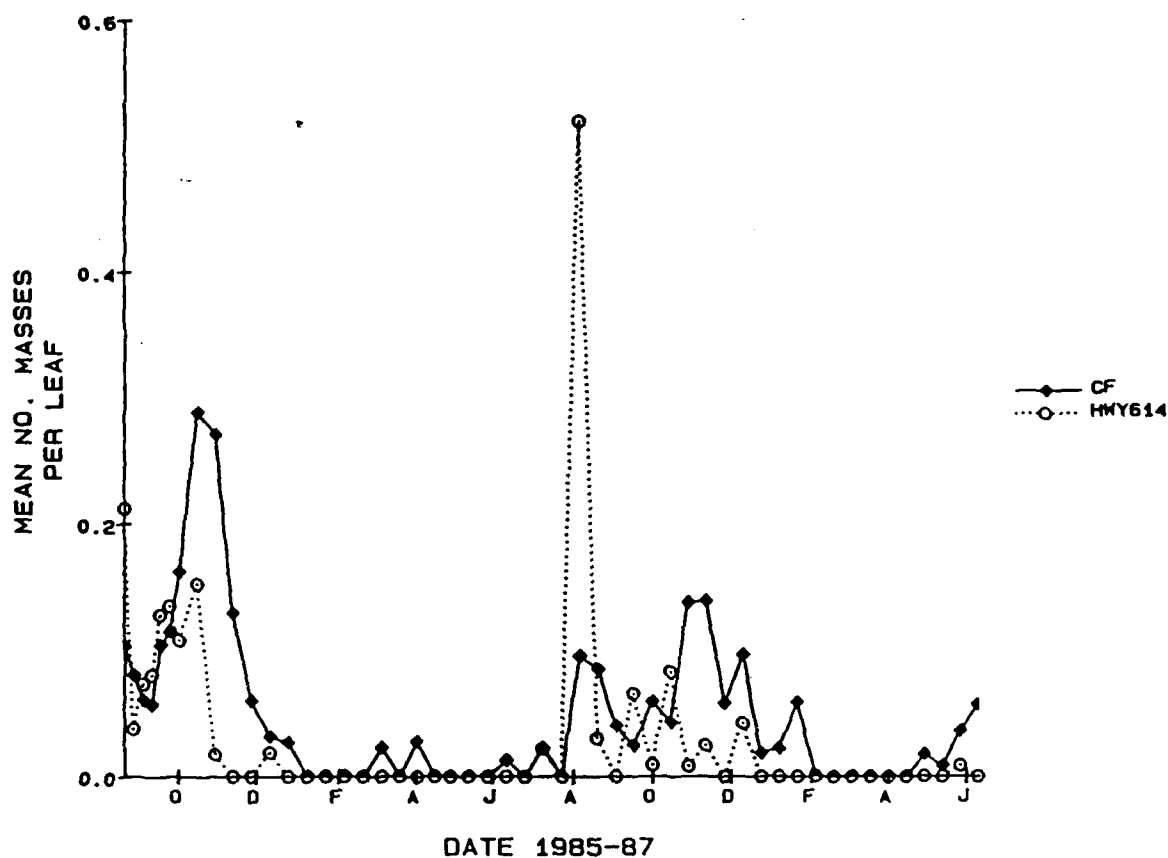


Fig. 16. Seasonal densities of viable Mansonia egg masses on P. stratiotes leaves at two sites. Approximately 100 leaves > 10 cm long were examined from ca ten plants during each survey.

614, respectively. Based on head capsule width measurements of first instar larvae from the first 146 egg masses collected, 87% were M. dyari and 13% were M. titillans.

The average abundances of larvae and pupae per quadrat sample were smaller at HWY 614 than CF on all sample dates but one (Fig. 17), the unique interval in early September of higher density at HWY 614 following the unusual peak in oviposition observed at the same site in late August (Fig. 16). The seasonal pattern of larval abundance was similar at both sites, post-winter decreases followed by recrudescences in the summer and maximal densities in the autumn or winter. Comparisons of 1986 and 1987 showed that larval densities were depressed less in the latter year's winter. The seasonal changes in larval density closely paralleled the temporal changes in leaf areas of P. stratiotes (Figs. 5 & 6).

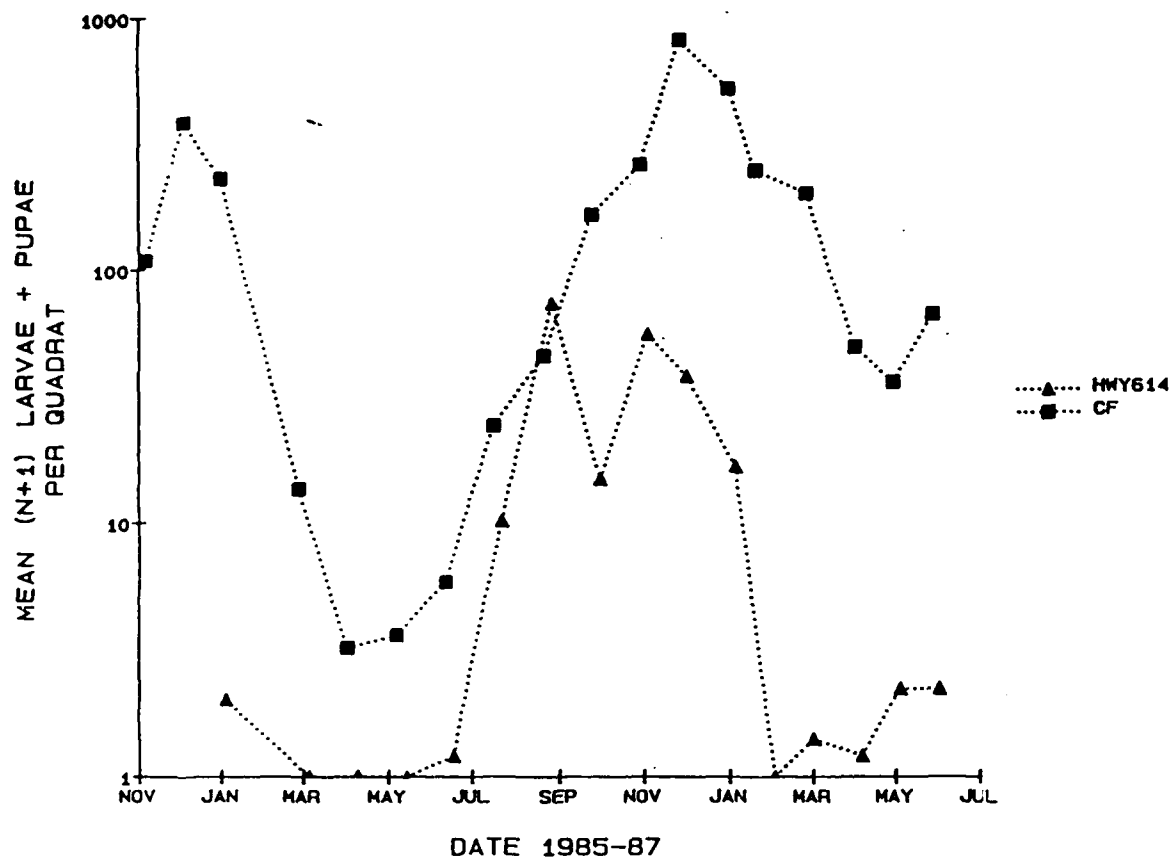


Fig. 17. Seasonal densities of larval and pupal *Mansonia* recovered per quadrat sample of *P. stratiotes*. Values are means of five quadrat samples.

The average numbers of adult *Mansonia* were highest during the first two months of emergence trapping and never returned to these levels in the following 1.5 years (Fig. 18). There is reason to suspect that the low catches were partly due to predation within traps. Seasonal fluctuations in abundance, so far as discernible, showed that most adult emergence occurred in the latter half of 1986, although there was evidence of a spring emergence following the milder winter in 1987. The percentage of *Mansonia* adults identified as *M. titillans* was significantly higher at HWY 614 ( $45/157 = 28.7\%$ ) than at CF ( $52/2003 = 2.6\%$ ) ( $957.72 = G_{adj} \gg \text{chi-squared}_{.001} = 10.83$ ).

#### Oviposition site selection

The temporal occurrence of upper and lower egg masses was compared at both sites, but there were no indications of seasonal trends in the frequencies of use of the two surfaces (Fig. 19). At CF, egg masses were predominantly on upper leaf surfaces ( $162/265 = 61.1\%$ ) whereas at HWY 614 egg masses were most common on under surfaces ( $100/160 = 62.5\%$ ). These frequencies are significantly different ( $36.11 = G_{adj} \gg \text{chi-squared}_{.001} =$

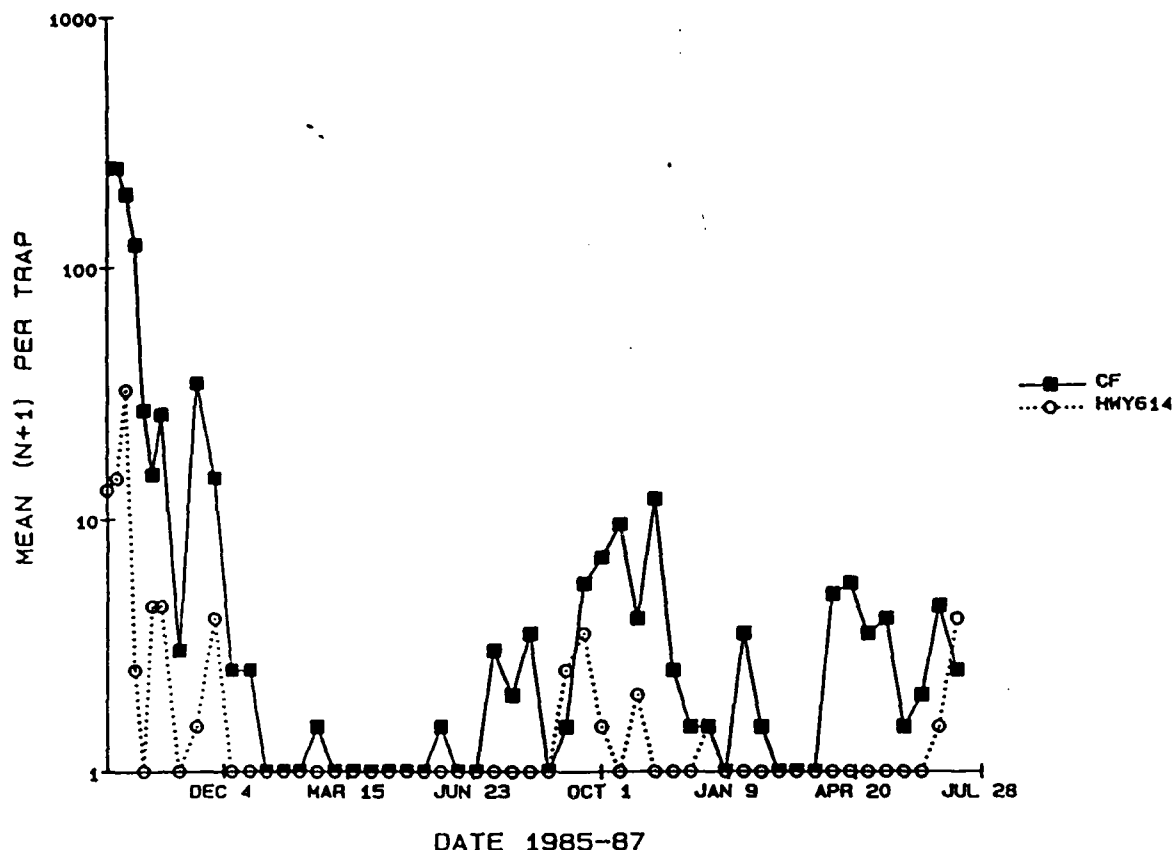


Fig. 18. Seasonal densities of adult *Mansonia* caught in two emergence traps, each covering 1 m<sup>2</sup> of water lettuce. Each collection covers a one-week period. <sup>2</sup>

10.83). The high under-surface frequency at HWY 614 was heavily influenced by the collection on August 20, 1986 when 84.6% (44/52) of the egg masses found were on lower leaf surfaces.

Among 207 viable *Mansonia* egg masses whose positions on leaves were mapped, 35.3% were on the under surfaces. All undersurface masses were within 0.25 cm of the leaf edge except for four which were laid apparently through holes caused by herbivorous insect damage (Fig. 20). The location of masses suggests that under-surface oviposition is performed with the female secured by mid- and hind legs to the upper surface, bending her abdomen into the water to reach the underside (22).

The remaining 134 egg masses (64.7%) were found on upper leaf surfaces. Prior to this report, only anecdotal information (23) had ever documented upper-surface oviposition by *Mansonia*. Upper-surface masses were usually located near the water line at the proximal end of the leaf, but were evenly distributed across the breadth of this line (Fig. 21). Many egg masses were recovered well above the water line, which would seem to strand larvae hatching in such locations.

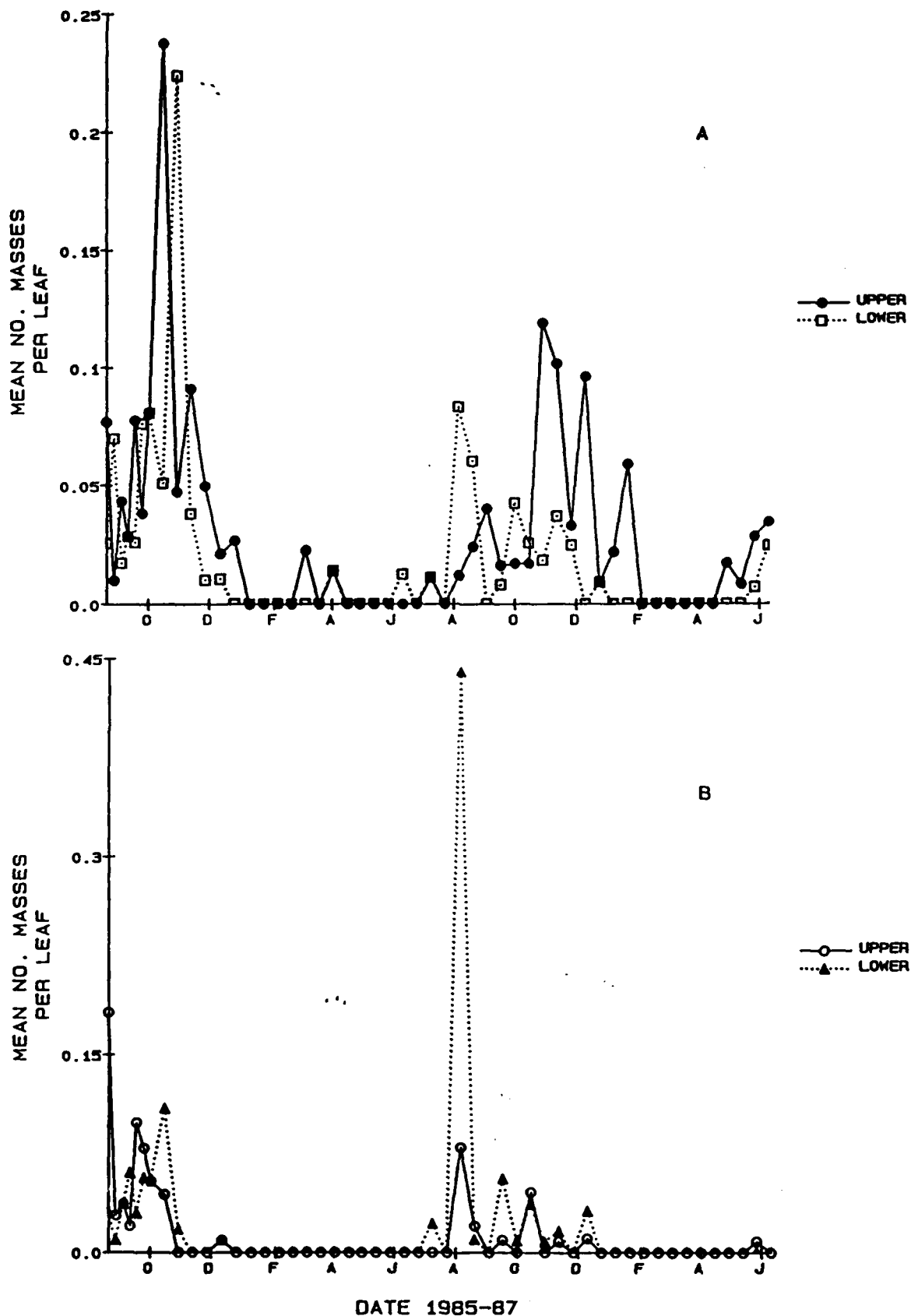


Fig. 19. Seasonal abundances of *Mansonia* egg masses on the upper and under surfaces of *P. stratiotes* leaves at (A) CF and (B) HWY 614. Approximately 100 leaves were examined for each survey point.

## LOWER LEAF

n = 73 EGG MASSES

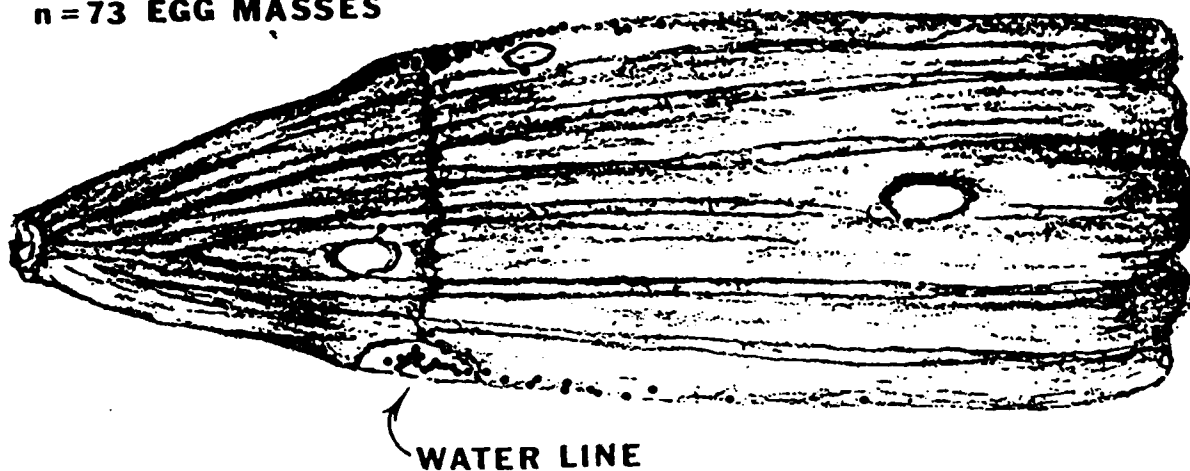


Fig. 20. The spatial distribution of *Mansonia* egg masses on the under surfaces of *P. stratiotes* leaves sampled between September 1985 and January 1986 during which time 3,257 leaves were examined. Each black dot represents a single egg mass. Ellipses are holes caused by insect damage through which four masses were laid.

## UPPER LEAF

n = 134 EGG MASSES

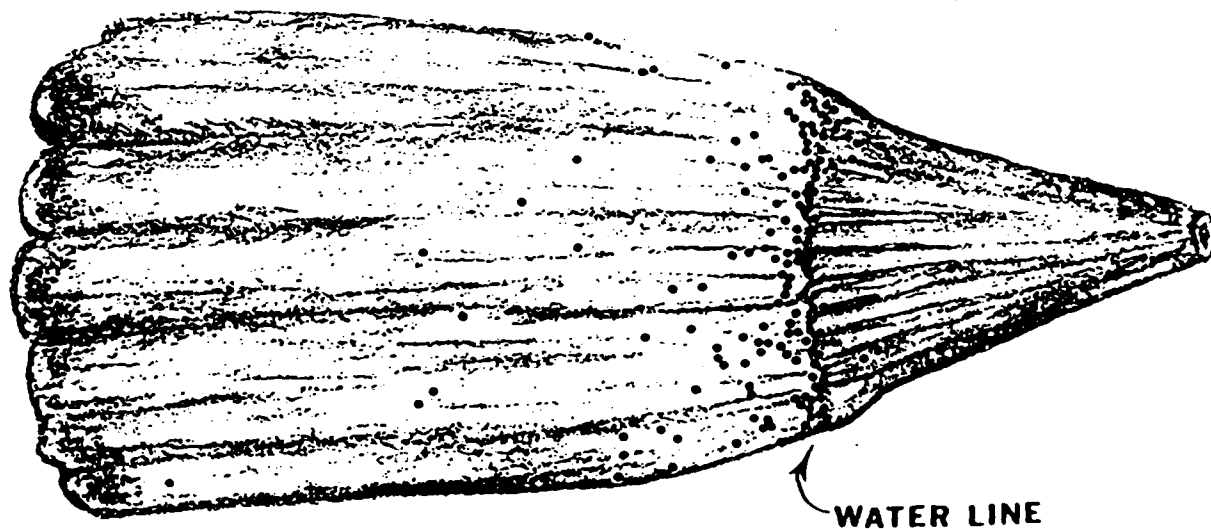


Fig. 21. The spatial distribution of *Mansonia* egg masses on the upper surfaces of *P. stratiotes* leaves sampled between September 1985 and January 1986. Each dot represents a single egg mass.

As many as five viable egg masses were recovered from single leaves among the 9,621 examined from nature (Fig. 22). The frequency distribution of egg masses per leaf was significantly different from a Poisson (random) distribution ( $347.92 = \text{Gadj. } \chi^2$  chi-squared (.001)(2df) = 13.82). The variance/mean ratio (coefficient of dispersion) was far greater than one, indicating clumping (overdispersion) of egg masses.

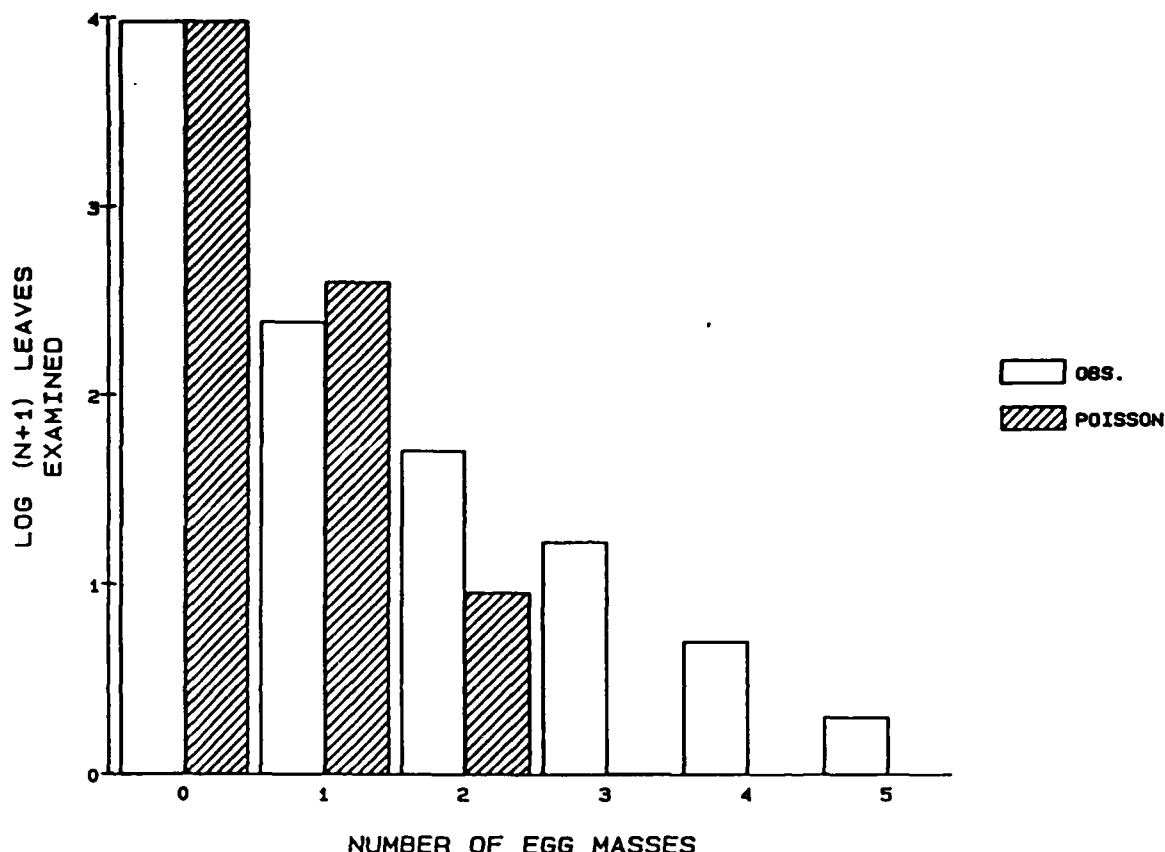


Fig. 22 The frequency distribution of number of egg masses per leaf from fortnightly surveys (n = 9,934 leaves examined) compared to an expected distribution based on a Poisson.

In three separate trials with gravid M. dyari confined in cages, 67 to 74 % of egg masses were laid on the upper surfaces of leaves of intact P. stratiotes plants (Table II). By contrast, in a single trial with M. titillans, no egg masses were deposited on upper leaf surfaces. These experiments confirm that M. dyari possesses a behavioral flexibility, absent in the related M. titillans, in oviposition site selection on P. stratiotes.

To examine how leaf orientation influenced oviposition site selection by M. dyari, gravid females were exposed overnight to a tray of excised P. stratiotes leaves which floated flat on the water surface. Under these conditions, all egg masses were laid on leaf undersides (Table III). These results suggest that M.

Table II. Oviposition of two species of *Mansonia* on whole *P. stratiotes* plants in cages.

Species	Trial no.	No. leaves exam.	No. upper masses	No. under masses
<i>M. dyari</i>	1	58	20	8
<i>M. dyari</i>	2	52	12	6
<i>M. dyari</i>	3	144	38	12
<i>M. titillans</i>	1	A	0	9

A = total no. of leaves not recorded

Table III. Oviposition of two species of *Mansonia* on floating *P. stratiotes* leaves in cages.

Species	No. trials	No. leaves with masses	No. upper	No. lower
<i>M. dyari</i>	1	8	0	22
<i>M. titillans</i>	1	5	0	5

*dyari* may use some cue associated with leaf orientation, such as angle from the horizontal, to decide upon upper versus lower oviposition. As expected, all *M. titillans* masses were found on the undersides of leaves.

Egg masses of *M. dyari* incubated in the laboratory required an average of eight days from oviposition to hatch at 26 C and between 15 and 16 days at 20°C (Table IV). If the long incubation time of eggs allowed masses originally oviposited above the water line to subside towards the water as the leaf ages (Fig. 15), a correlation between egg mass age and leaf angle from the horizontal might be detectable. Angles of leaves which held egg masses were measured at CF, and these masses brought to the laboratory for incubation at 26°C until hatching. There was no significant correlation (product-moment  $r$ ) between leaf angle and number of days to hatch (Table V). Neither mean times to hatch nor mean leaf angles of upper versus lower egg masses were significantly different ( $t = 0.25$  &  $-1.59$ , both ns), suggesting that upper or under surface oviposition may be determined only after the appropriate leaf has been chosen.

Table IV. Egg incubation times for *Mansonia* at two temperatures.

	<u>Number of days from oviposition until hatch</u>						
Temp. (C)	7	8	9	.....	15	16	17
20					7	1	4
26	1	7	1				

Table V. Association of leaf angles from the horizontal and developmental times of egg masses.

	<u>No.</u>	<u>mean angle (s.dev.)</u>	<u>mean days (s.dev.) to eclosion</u>	<u>correlation (r)</u>
Upper masses	54	28.0 (15.3)	5.9 (2.3)	0.19 (ns)
Under masses	41	33.2 (14.7)	5.7 (3.6)	-0.02 (ns)

The survivorship of larvae which hatched out of water was inversely related to the time between hatch and egg mass submersal. Larvae which remained sequestered in their egg shells just one day after hatching showed negligible mortality, but survival dropped dramatically after two and three days of post-hatch maintenance out of water (Table VI).

Table VI. Survivorship of M. dyari larvae experiencing delayed eclosion.

<u>Hours delayed</u>	<u>No. of masses</u>	<u>Larval survival (%)</u>	<u>No. masses with total mortality</u>
24	8	97.8	0
48	11	15.8	5
72	9	7.2	5

#### Insects from Pistia roots

Identifications of entomofauna recovered in quadrat samples are tabulated in Appendix I. For the 73 quadrat samples scored from CF, the number of individuals of each insect order was tallied. Diptera were most abundant, followed in sequence by Odonata, Hemiptera and Odonata (Fig. 23). Aquatic Lepidoptera and Ephemeroptera comprised much smaller proportions of the total.

When length measurements from collected specimens were used to estimate biomass from the length versus dry weight regressions of Smock (1980), the rank order of importance of the six commonest orders was altered (Fig. 24). Odonata contributed an order of magnitude more biomass than Diptera, the former accounting for 77.0% and the latter 12.8% of the insect dry weight associated with P. stratiotes roots. Coleoptera ranked third in biomass, but the contribution of this order was only 9.5% of the total estimate.



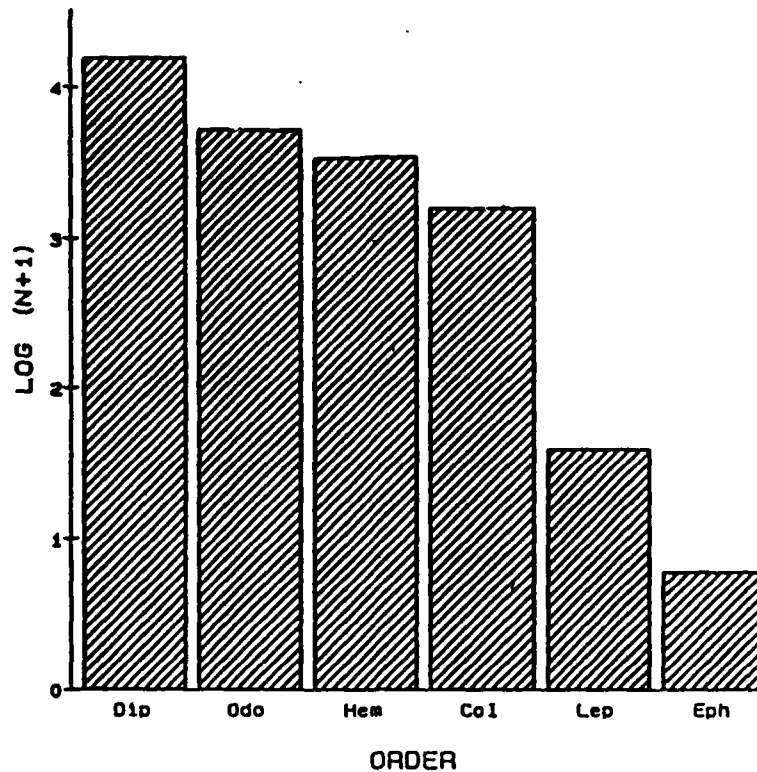


Fig. 23. Total abundances of number of individual insects of the six commonest orders taken in monthly quadrat samples ( $n = 73$ ) at CF between November 1985 and March 1987. Abbreviations: Dip = Diptera, Odo = Odonata, Hem = Hemiptera, Col = Coleoptera, Lep = Lepidoptera and Eph = Ephemeroptera.

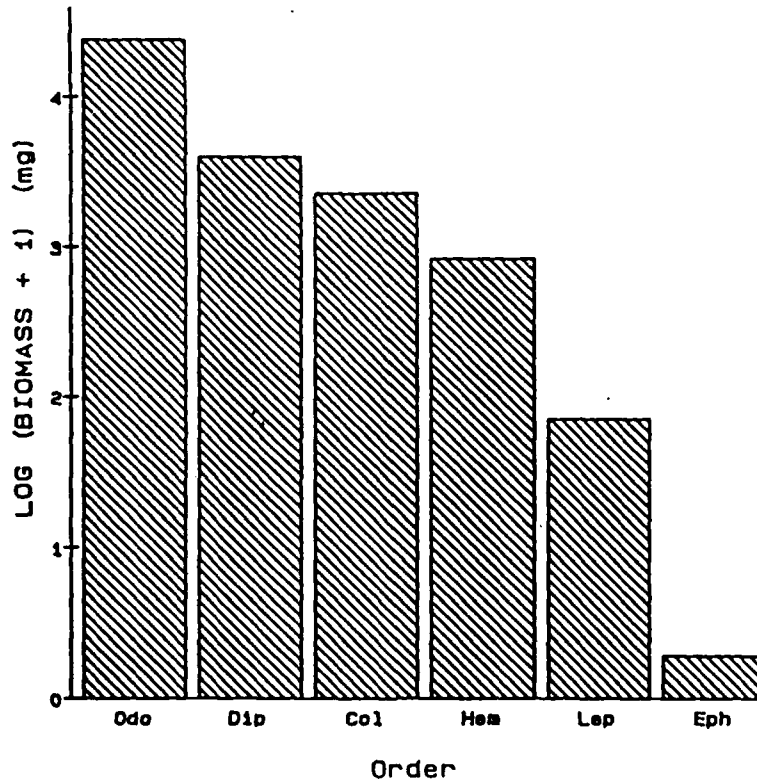


Fig. 24. Estimated biomasses of commonest insect orders taken in monthly quadrat samples ( $n = 73$ ) at CF between November 1985 and March 1987. Dry weights were estimated from length measurements and length versus weight regressions in Smock (1980). Abbreviations as in Fig. 23.

The mean number of aquatic insect species per quadrat was compared among eleven successive sample dates at CF. Species richness ranged from 13 to 17 per quadrat and was lowest in October and highest in December (Fig. 25). The cause of the decrease in number of species during October is unclear, although the fewest numbers of Odonata were recorded in that month (Fig. 26).

Because of their apparent important contribution to the invertebrate community on *P. stratiotes* roots and our ability to recognize individuals by species, particular attention was devoted to the Odonata in CF quadrat samples. Seasonally, the numbers of individual odonates per quadrat peaked in the winter, maxima observed in January 1986 and February of 1987 (Fig. 26). The average number of individuals per quadrat was lowest from April through October of 1986.

Examination of size class frequencies of the most abundant odonate species, the zygopteran *Telebasis byersi*, revealed that periods of relatively high odonate abundance such as December 1986 through February 1987 were characterized by the numerical dominance of the smallest size class (Fig. 27). However, by April

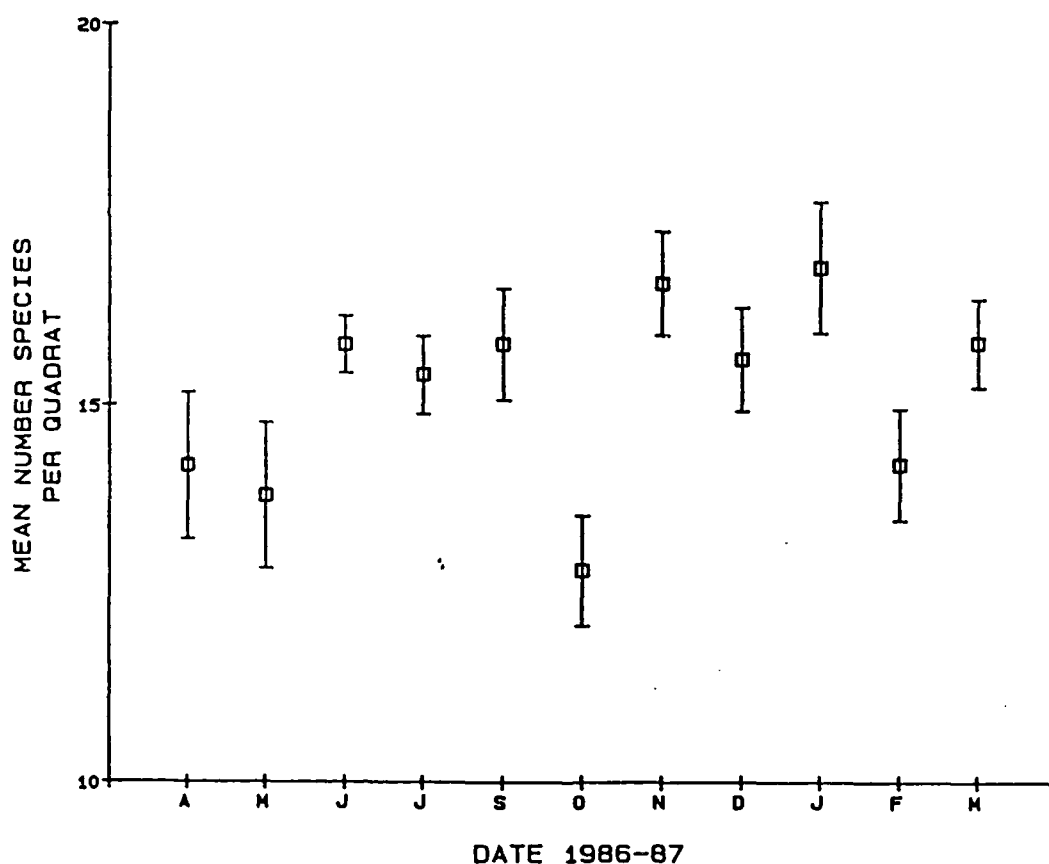


Fig. 25. Seasonal variation in species richness of aquatic insects from quadrat samples at CF. Vertical bars denote  $\pm 1$  s.e.

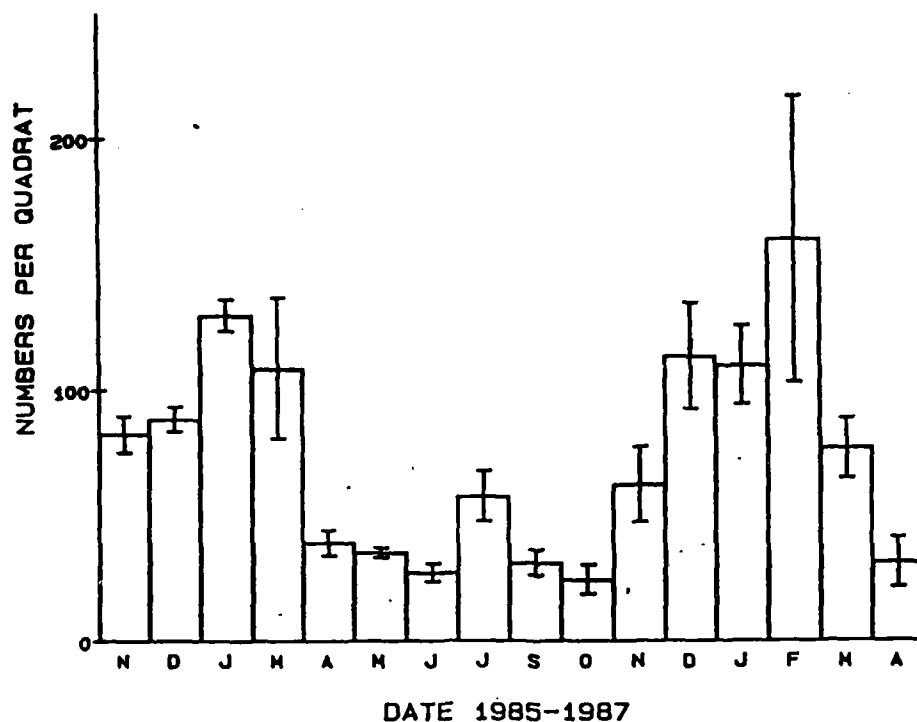


Fig. 26. Seasonal abundance of larval Odonata in monthly quadrat samples at CF. Vertical lines denote  $\pm 1$  s.e.

these smallest nymphs were no longer the commonest size class, and the total reduction in odonate numbers during the spring pointed towards prominent mortality among early instars of T. byersi. The occurrence of the largest size class only during the spring suggested that this species is univoltine in our area.

Although the average number of Zygoptera exceeded that of Anisoptera because of the numerical dominance of T. byersi, Anisoptera contributed more to insect biomass in quadrats (Table VII) owing to their larger size. For example, the largest Anisopteran, Coryphaesna adnexa, accounted for only 1.6% of the number of odonates per square meter, yet 21.9% of the total odonate biomass.

Table VII. Abundance measures and biomass estimates of seven larval Odonata recognized from quadrat samples at Chinese Farm.

Species	Mean no./m <sup>2</sup>	S.E.	Biomass (mg)
<u>Erythemis simplicicollis</u>	33.78	3.68	1947.37
<u>Pachydiplax longipennis</u>	27.30	3.09	4252.49
<u>Coryphaeschna adnexa</u>	7.87	1.63	3744.48
<u>Miathyria parcella</u>	5.50	0.60	370.50
<u>Telebasis byersi</u>	156.95	19.14	5771.52
<u>Ischnura posita</u>	12.13	2.14	482.41
<u>Ischnura rambui</u>	8.00	2.54	512.93

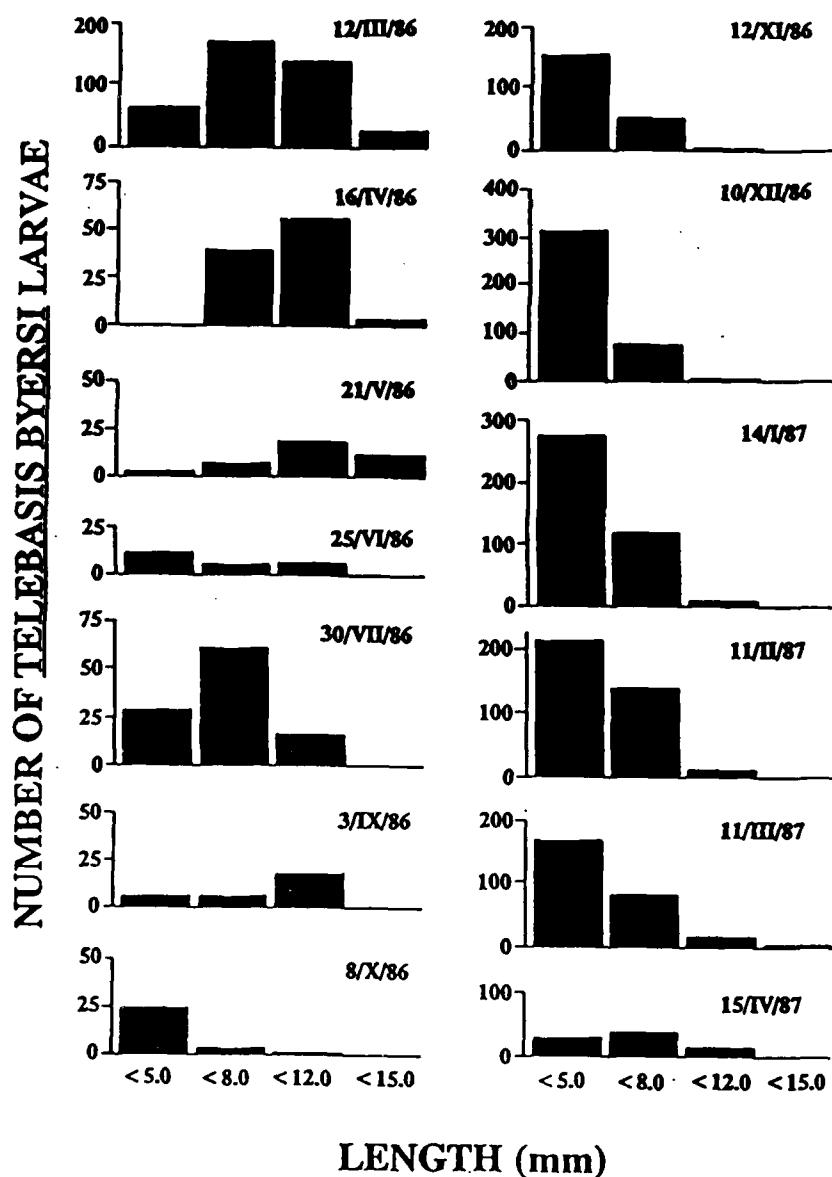


Fig. 27. Size frequency distributions of the zygopteran *Telebasis byersi* in monthly quadrat samples from CF. Each date shows the total larval abundance in five quadrat samples except March and April 1986 when four samples were taken.

#### Predation on *Mansonia* larvae

Examination of the foreguts of both Zygoptera and Anisoptera collected from CF revealed evidence of odonate predation upon *Mansonia* larvae. It was possible to discern the remains of identifiable invertebrates in 77 odonate guts and of these, 20.7% contained parts of *Mansonia* exoskeleton (Table VIII).

Fish were captured from two separate CF ponds by dipping with a bucket or with a Hart trap set for four days. In both cases, dissection of the fish revealed *Mansonia* exoskeletal remains in the guts of some *G. affinis*, but none in *H. formosa*.

Table VIII. Surveys of odonates from CF for Mansonia remains in dissected guts.

Collection date	Pond No.	Odonata	Median len. (mm) (range)	No. with Inverts	No. with <u>Mansonia</u>
I/29/86	A8	10 Zygoptera 10 Anisoptera	12 (10-15) 7.5(5-26.5)	10 10	1 3
I/31/86	A10	7 Zygoptera 7 Anisoptera	9.5(8-10) 9 (7-15.5)	3 6	1 0
XI/17/86	A8	16 Zygoptera 13 Anisoptera	6 (4-11) 9.5(5-18)	6 10	1 2
I/5/87	A8	77 Zygoptera 9 Anisoptera	7 (3-13) 9 (4-19)	24 8	6 2

(Table IX). Overall, 43.5% of the G. affinis with identifiable invertebrates contained pieces of Mansonia exoskeletons in their guts.

During January and February of 1987, 17 ponds with P. stratiotes were surveyed for the abundance of Mansonia larvae and pupae by censusing the mosquitoes on the roots of three plants. A range of one to 708 larvae and pupae were recovered per pond (Table X). The fish populations in each pond were censused with a single Hart trap. A range of 0 to 48 G. affinis were caught per trap with a median number of 17. Although up to 45 H. formosa were caught in a pond, the median of this species was zero. Because of the evidence, reported above, of G. affinis predation on Mansonia larvae, we attempted to correlate numbers of collected Mansonia and G. affinis with a rank-order coefficient. A calculated negative coefficient was not statistically significant (Spearman's  $\rho = -0.31$ ,  $t_s = 1.26$ , 18 df).

Studies of predation by fish on Mansonia larvae attached to Pistia in aquaria confirmed the impact of G. affinis and the relative inefficacy of H. formosa. At ratios of 1 G. affinis to 10-13 Mansonia, 65 to 95 % of larvae were 'missing' from roots 24 hours after the initiation of the experiment (Table XI). By contrast, one H. formosa for each eight Mansonia reduced mosquito numbers by only 29% during the same observation period.

Table IX. CF fish dissected for stomach contents

Collection date	Pond/ method	No. of <u>Mansonia</u> larvae on five plants					Fish examined	No. with Inverts.	No. with <u>Mansonia</u>
		I	II	III	IV	P			
I/9/87	B4/ bucket	21	65	99	41	1	12 <u>G. affinis</u> 8 <u>H. formosa</u>	12 0	7 0
II/5/87	C3/ Hart	7	213	284	202	2	16 <u>G. affinis</u> 1 <u>H. formosa</u>	11 0	3 0

Table X. Survey of 17 CF ponds for Mansonia and fish, January - February 1987.

Pond	Number of <u>Mansonia</u> on three plants						Number of fish caught in Hart trap	
	I	II	III	IV	P	Tot.	<u>G. affinis</u>	<u>H. formosa</u>
A2	0	0	0	2	0	2	17	14
A3	0	0	0	1	0	1	25	45
A4	3	1	2	2	0	8	0	1
A5	0	4	0	0	0	4	6	7
A7	2	17	54	113	1	187	0	0
A8	47	129	126	349	2	653	0	0
A13	1	16	2	3	0	22	24	7
A14	1	2	2	3	0	8	18	0
B2	0	1	0	2	0	3	46	0
B3	0	3	1	1	0	5	25	0
B4	3	30	42	60	0	135	12	3
B8	2	6	3	4	0	15	34	0
B9	17	28	18	10	0	73	48	0
C1	6	38	15	6	0	65	11	0
C2	1	11	10	4	0	26	15	0
C3	7	213	284	202	2	708	17	5
C4	5	18	19	5	0	47	29	6

Table XI. Fish predation upon Mansonia larvae attached to P. stratiotes in aquaria<sup>A</sup>.

Exper. no.	No. plants	Orig. no. <u>Mansonia</u> <sup>AA</sup>	No. of fish		<u>Mansonia</u> remaining after 24 h	% loss
			<u>G. affinis</u>	<u>H. formosa</u>		
1a	2	100	10	-	5	95
b	1	50	5	-	3	90
c	1	50	-	-	50	0
2a	1	80	5	-	28	65
b	1	80	-	-	76	5
3a	1	80	-	10	57	29
b	1	80	-	-	75	6

<sup>A</sup> 80-litre aquaria containing large plants in filtered pond water. Larvae allowed 24 hr to attach before fish were introduced.

<sup>AA</sup> III + IV instars

Odonate larvae collected in quadrat samples were isolated with known numbers of Mansonia larvae attached to roots in beakers or aquaria. Limited predation by T. byersi or P. longipennis was inferred from Mansonia missing in beakers (Table XII). Larger numbers of both odonate larvae and potential prey increased probable predation, and Mansonia remains were detected in dissected guts of T. byersi, E. simplicicollis and C. adnexa. However in one trial, the reduction in Mansonia abundance in an aquarium with odontates was no less than in a control without predators (Table XII).

### Larval dispersal

The sampling regimen of Bailey (21) was repeated to substantiate his claim that nocturnal collections of Mansonia larvae yielded significantly fewer larvae than diurnal collections from the same habitat. Ten plants collected at 2100 h

Table XII. Odonate predation upon Mansonia larvae attached to P. stratiotes roots in vessels.

Vessel	Predators	No. of <u>Mansonia</u>	time exp.(hr)	No. <u>Mansonia</u> remaining	Gut content analysis of odonates
1000 ml beaker	8 <u>T. byersi</u>	7	48	3	no <u>Mansonia</u> remains
1000 ml beaker	2 <u>P. longipennis</u>	5	90	3	-
	control	5	90	5	
aquarium	2 <u>C. adnexa</u> 6 <u>P. longipennis</u> 5 <u>E. simplicicollis</u> 6 <u>T. byersi</u> 3 <u>Ischnura</u> sp.	170	48	113	<u>Mansonia</u> in guts of 1 <u>E. simplicicollis</u> & 1 <u>T. byersi</u>
aquarium	1 <u>C. adnexa</u> 2 <u>E. simplicicollis</u> 4 <u>P. longipennis</u>	100	48	74	<u>Mansonia</u> in gut of <u>C. adnexa</u>
	control	100	48	72	

yielded a mean of  $43.4 \pm 25.2$  (s.d) larvae while ten additional plants collected at 0900 h the following morning harbored  $17.1 \pm 9.1$  (s.d) larvae per plant. Nocturnal collections were significantly larger at  $P = 0.07$  ( $t = 1.91$ , 18 df), which does not support Bailey's results or hypothesis of nocturnal larval dispersal.

The placement of cleaned plants in ponds to test for dispersal and colonization by larvae yielded mixed results. In pond A3 which had background densities of ca 100 larvae per plant, there was little evidence of recruitment one or four days after setting cleaned plants (Table XIII). However when natural densities neared 400 larvae per plant in pond A7, migration of larvae to cleaned plants was observed at one, four, or eight days after setting. Larval densities on the experimental plants at four or eight days exceeded background densities (Table XIII). Most larvae on experimental plants were later instars, substantiating that colonization had occurred by larval dispersal from other roots rather than by hatching. When this experiment was repeated undersimilar conditions in the same pond one month later, results were dramatically different. In this second experiment at A7, there was only slight evidence for colonization of cleaned plants eight days after setting, recruitment being far below that observed in the previous trial.

Table XIII. Colonization of cleaned P. stratiotes plants by Mansonia larvae.

Date exper.	CF Pond	Background <u>Mansonia</u> density ( $\bar{x} \pm SE$ , $n=5$ )/plant	Avg. instar No. (AIN)	Mean no. recovered after:			
				1h	1d	4d	8d
IX/86	A3	$95.8 \pm 26.8$	3.0	0.8	0.2	-	-
IX-X/86	A3	$101.4 \pm 51.5$	2.8	0.8	-	2.2	-
XI/86	A7	$396.4 \pm 238.0$	3.0	11.6	72.2	481.2	588.0
XII/86	A7	$316.6 \pm 120.1$	2.6	16.6	1.2	5.6	29.2

## VI. Discussion and Conclusions

The seasonal growth of P. stratiotes at our study sites was similar to the phenology of another common floating macrophyte Eichhornia crassipes (water hyacinth) in a north-central Florida lake (24). Both of these tropical aquatic plants were negatively affected by cold which depressed leaf (lamina) areas and plant biomass in the winter. In both species of macrophytes, spring regrowth was manifested by production of large numbers of small leaves. However, E. crassipes biomass decreased in the autumn, whereas during the same season P. stratiotes maintained or increased vernal standing crop levels. Center and Spencer (24) concluded that solar radiation and intraspecific competition were important factors determining the productivity of E. crassipes in Florida; the relative contributions of these variables to growth of P. stratiotes in south Florida is not clear.

Most measures of P. stratiotes growth at CF and HWY 614 fell within ranges observed for this species in a tropical lake (14). Unlike Florida, no biomass depressions due to winter cold were observed in the tropics, where diebacks have been attributed to depletion of nutrients (25) or viral disease (26). In both Ghana (14) and Florida, peak flowering occurred in December following periods of steady-state maintenance of high standing crops. At both sites, plant densities and RGRs increased at similar rates after dieback, regardless of the cause of decline.

The first two months of Mansonia egg surveys suggested that oviposition intensity was similar at CF and HWY 614. However, in the subsequent twenty months egg mass densities at CF exceeded HWY 614 on all but three sampling occasions. The difference between larval densities at the two sites was even greater than egg densities, possibly indicating that larval mortality may be higher at HWY 614. Some larvae were recovered at CF on all sampling dates, whereas the local larval populations at HWY 614 were completely depleted after winter cold in both 1986 and 1987. Almost twenty times the number of Mansonia adults were captured in emergence traps at CF compared to HWY 614, although the absolute numbers in traps did not accurately measure the emergent mosquito population. The differences in relative abundance of M. titillans compared to M. dyari at the two sites may indicate that local conditions at CF and HWY 614 differ in their suitability for the two species.

Among measures of water quality variables, CF and HWY 614 differed significantly only in pH and total phosphates. The average pH at both sites was near neutral, which is believed to be optimal for P. stratiotes (27) in spite of one report that water lettuce favors acidic conditions (28). Phosphorus levels at both sites were above 0.50 - 1.0 mg/l believed to be the threshold required for floating macrophytes (24), and sufficient nitrogen should have been available from ammonium, the major source of this element for freshwater macrophytes (29).



What, then, may be the causes of the differences in productivity of M. dyari at CF compared to HWY 614? One obvious factor is the lower P. stratiotes biomass, and hence reduced substrate, at HWY 614 compared to CF. Although both sites experienced reductions in water lettuce density after winter cold, the diminution at HWY 614 was more severe, causing virtual elimination of the root substrate for three months in 1986. While local extinctions may be temporary, it may take M. dyari multiple generations to rebuild to abundance levels observed prior to habitat disruption. Mansonia dyari are not vagile mosquitoes as adults (30), and weak adult dispersal would further delay recolonization of depleted habitats.

Predation may also keep M. dyari abundance levels lower at HWY 614. Fish were extremely common at HWY 614, although no population estimates were done to compare the two sites. Evidence presented elsewhere in this report indicates that mosquitofish, G. affinis, have the potential to regulate M. dyari populations.

The specificity of oviposition site selection by phytophagous insects on plants has been the subject of many recent investigations (31). Herbivorous insects specific to floating macrophytes also show great selectivity in oviposition site selection (32). Most previous field studies on oviposition by Mansonia focused on egg mass aggregation and gross features of favored habitats (33-35) instead of mapping egg mass locations on plants. The research presented in this report is the first recognition of an ovipositional specialization by Mansonia particularly adapted to egg placement on emergent floating vegetation. The discovery of upper surface oviposition by M. dyari on P. stratiotes is the first report of such behavior in Mansonia, except for an anecdotal note by Boreham (23) who recovered egg masses of M. lebereri on both upper and lower surfaces of water lettuce leaves in Panama.

There are several lines of evidence which suggest that upper-surface egg laying is a specialization of M. dyari for P. stratiotes. Firstly, M. titillans, a generalist Mansonia which uses host plants other than water lettuce, cannot lay upper surface masses. Secondly, M. dyari oviposits preferentially on P. stratiotes leaves which are destined to submerge by the time hatching should occur.

In spite of the synchrony between egg development and leaf senescence, there is still some risk of desiccation to egg masses laid out of water, especially if leaf drooping is delayed by support of crowding neighboring plants. Since larval hatch out of water produces high mortality, there should be some advantage to upper surface oviposition that counterbalances the risk of desiccation. In view of the abundance of aquatic predators associated with P. stratiotes roots, eggs laid out of water may be more protected from attacks by carnivorous invertebrates.

Examination of the invertebrates inhabiting P. stratiotes

roots revealed that, although Diptera were numerically dominant, Odonata accounted for a far larger proportion of the biomass than any other insect order. The numerical dominance of Diptera and biomass dominance of Odonata had previously been observed for the invertebrate community on water lettuce in Ghana (36).

Since the work of Van den Assem (37), it has been assumed that Mansonia larvae and pupae escape intense predation because of their attachment to roots. However, Van den Assem examined a very narrow range of predators. The current report is the first to describe significant predation of Mansonia larvae and pupae in nature. The most important predator at our field sites seems to be G. affinis. Although larvivorous fish are the most widespread and effective biocontrol agents used against mosquitoes (38), they are not deployed to control Mansonia (39). Our report is also the first to document predation by Odonata on Mansonia in nature, although dragonfly larvae have long been known to include some mosquitoes in their diets (40). For both G. affinis and Odonata, it will be important to examine in future studies how these predators locate Mansonia prey attached to roots.

If mature Mansonia larvae periodically leave roots to disperse to other sites, they might be more susceptible to predation during detachment. Experiments reported herein cast doubt upon the postulated nocturnal dispersal of larvae (24). The tremendous recruitment to 'cleaned' P. stratiotes plants seen in one experiment indicates that some interplant movement does occur, but at present the stimuli and timing of larval dispersal need to be resolved by further investigations.

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Appendix I. List of invertebrates and fish captured in quadrat samples at CF and HWY 614. Where genus and species are not given, identification was made only to family level.

Mollusca  
Gastropoda  
Pulmonata  
Lymnaeidae  
Lymnaea sp.  
Planorbidae  
Planorbis gyratus

Pelecypoda  
Family unknown

Platyhelminthes  
Turbellaria  
Tricladida  
Planariidae

Annelida  
Oligochaeta  
Family unknown

Hirundinea  
Rhynchobdellida  
Glossiphoniidae  
Placobdella rugosa

Arthropoda  
Crustacea  
Ostracoda  
Family unknown

Copepoda  
Family unknown

Amphipoda  
Talitridae  
Hyalolella azteca

Decapoda  
Family unknown

Insecta  
Diptera  
Culicidae  
Mansonia dyari  
Mansonia titillans  
Culex erraticus  
Culex sp.  
Anopheles sp.  
Uranotaenia sp.  
Cogulilettidia perturbans

Chaoboridae

Diptera (continued)  
Ceratopogonidae

Chironomidae

Stratiomyidae  
Hedriodiscus trivittatus  
Tabanidae

Ephemeroptera  
Caenidae  
Caenis sp.

Odonata  
Libellulidae  
Pachydiplax longipennis  
Miathyria marcella  
Erythemis simplicicollis

Aeshnidae  
Coryphaeschna adnexa

Agrionidae  
Telebasis bryersi  
Ischnura posita  
Ischnura rambui

Hemiptera  
Pleidae  
Plea striola

Naucoridae  
Pelocoris femoratus

Belostomatidae  
Belostoma fuminea  
Lethocerus sp.

Corixidae

Notonectidae

Naucoridae

Nepidae  
Ranatra sp.

Hebridae

Mesoveliidae

Coleoptera  
Halipidae

Peltodytes sexmaculatus  
Peltodytes floridensis

Dytiscidae  
Celina slossoni  
Hydroporus lynceus  
Liodessus affinis  
Desmopachia grana  
Hydaticus bimarginatus  
Laccophilus proximus  
Copelatus caelaticollis  
Hydrovatus pustulatus

Hydrophilidae  
Neohydrophilus castus  
Enochrus pygmaeus  
Tropisternus blatchleyi  
Paracymus nanus  
Paracymus sp.  
Tropisternus lateralis

Helodidae

Noteridae  
Hydrocanthus oblongus  
Suphisellus insularis  
Hydrocanthus regius  
Suphis inflatus  
Suphis puncticollis

Carabidae

Lathrididae

Neuroptera  
Sialidae

Lepidoptera  
Tineidae  
Acrolophus sp.

Pyrallidae  
Samea sp.

Arachnida  
Acarina  
Hydrachnellae

Vertebrata  
Osteichthyes  
Poeciliidae  
Gambusia affinis  
Heterandria formosa

Appendix II. List of insects captured in emergence traps at CF and HWY 614. Where identifications are given only at the family level, genus and species determinations are not yet available.

# Diptera

## Culicidae

Culex erraticus  
Mansonia dyari  
Mansonia titillans  
Anopheles crucians  
Anopheles quadrimaculatus  
Uranotaenia lowii  
Uranotaenia sapphirina  
Culex nigripalpus  
Coquillettia perturbans

## Ceratopogonidae

Atrichopogon cf. levis  
Atrichopogon cf. websteri  
Bezzia glabra  
Culicoides insignis  
Dasyhelea mutabilis  
Dasyhelea cf. grisea  
Forcipomyia sp.  
Forcipomyia calcarata  
Stilobezzia bulla  
Stilobezzia coquilletti  
Stilobezzia sybleae

## Chironomidae

Chironomus decorus  
Goeldichironomus holoprasinus  
Monopelopia boliekae  
Parachironomus directus  
Polypedilum trigonus  
Tanytus punctipennis  
Tanytarsus sp.  
Ablabesmyia peleenis  
Limnophyes nudiradius  
Larsia declorata  
Paramerina testa  
Stempellina sp.  
Zavrelliella varipennis

## Sciaridae

Bradsia sp.

## Chaoboridae

Chaoborus sp.

## Stratiomyidae

Hedriodiscus trivittatus

## Psychodidae

Psychoda lativentris

## Syrphidae

Palpada agrorum

## Agromyzidae

## Cecidomyiidae

Karshomyia sp.  
Bremia sp.  
Diadiplosis sp.  
Mycodiplosis  
Resseliella sp.  
Didactylomyia longimana  
Leptodiplosis sp.  
Anarete sp.

## Chloropidae

## Dolichopodidae

Chrysotus sp.

## Spherooceridae

Leptocera sp.

## Ephydriidae

## Phoridae

## Tachinidae

## Tipulidae

## Lepidoptera

### Pyrilidae

Samea multiplicalis  
Synclita oblitalis

## Homoptera

### Cicadellidae

Empoasca sp.  
Draeculacephala mollipes

## Aleyrodidae

Trialeurodes abutilonea

## Aphididae

## Thysanoptera

### Phlaeothripidae

Hoplothrips flavicauda

## Hymenoptera

### Eulophidae

Tetrastichus sp.

### Braconidae

### Diapriidae

### Formicidae

### Trichogrammatidae

### Agaonidae

Parapristina verticillata

### Torymidae

Philotrypesis emeryi

### Pteromalidae

Odontofroggattia galili

## Ephemeroptera

### Caenidae

Caenis sp.

## Coleoptera

### Noteridae

### Dytiscidae

### Staphylinidae

Carpelimus sp.  
Thinobius sp.  
Euaesthetus sp.  
Euaesthetus atomus  
Neohypnus pusillus  
Neobisnius ludicrus  
Myllaena arcana  
Thecturota sp.

## Odonata

### Libellulidae

Erythemis simplicicollis  
Miathryia marcella

### Agrionidae

Telebasis byersi

**IX. Personnel Salaried by Project:**

L. B. DeWald (Biological Scientist) 1985-87

J. L. Fox (Technician) 1985-86

N. Nishimura (Technician) 1986-87

V. L. Shively (Technician) 1986

F. E. Vose (Craftsman) 1985



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